STUDIES ON THE FATTY ACID COMPOSITION OF HUMAN SERUM WITH SPECIAL REFERENCE TO THE INFLUENCE OF HORMONES AND OTHER AGENTS

A thesis submitted for the Degree of

Doctor of Philosophy

in the

University of Edinburgh

by

Jadwiga Jurand

APRIL, 1968

University Department of Clinical Chemistry
Royal Infirmary, Edinburgh

TABLE OF CONTENTS

PREFACE

One of the most important aspects of atherogenesis is the metabolism and transport of lipids, which are the major components of arterial plaques. Several studies have indicated that some of the plaque lipids are derived from plasma (Zilversmit and McCandless, 1959; Zilversmit, et al., 1961; Newman and Zilversmit, 1962). It has been assumed that the degree of deposition may, in part, depend upon the structure of the fatty acids (Sinclair, 1956).

It is well known that certain abnormalities in plasma lipids occur in patients with ischaemic heart disease. The correlation between high levels of plasma cholesterol and the incidence of ischaemic heart disease has been widely reported (The Cooperative Study of Lipoproteins and Atherosclerosis 1956, Doyle et al., 1957; Keys et al., 1958; Epstein et al., 1959; Kannel et al., 1961). Increased plasma triglyceride concentrations have also often been observed (Albrink and Man, 1959; Antonis and Bersohn, 1960; Brown et al., 1965), and different types of hyperlipoproteinaemia seen in this disease have been described (Havel and Carlson, 1962; Fredrickson et al., 1967a.b.c.d.e.). Attention has recently been focussed upon the observation that patients with a history of myocardial infarction have increased levels of plasma free fatty acids (Kershbaum and Bellet, 1964; Rifkind, 1966)

and that these increases may be particularly marked immediately after the myocardial infarction (Kurien and Oliver, 1966).

Comparatively little is known about the role which structural differences between individual free fatty acids play in the development of hyperlipidaemia and ischaemic heart disease. Recent views on the role of plasma free fatty acids in the transport of lipids (Gordon and Cherkes, 1956; Dole, 1956) and in the pathogenesis of hyperlipidaemia (Rudman, 1963; Steinberg, 1963), as well as on their possible influence in the development of atherosclerotic vascular disease (Carlson et al., 1965) indicate that qualitative as well as quantitative considerations are of importance.

It is now well known that fatty acid mobilisation is controlled directly or indirectly by both neural and hormonal factors. The endocrine influences upon coronary atherogenesis considered earlier by Oliver and Boyd (1955, 1956, 1958, 1959) thus acquire new importance. The concept that fatty acid mobilisation is the "spiritus movens" in the transportation of lipids raises several questions:

1. Do different adipokinetic agents increase the concentrations of individual fatty acids according to a common pattern or specifically?
2. How are the changes in fatty acid mobilisation reflected in the fatty acid composition of serum triglycerides, phospholipids and cholesterol esters?

3. If such a relationship exists, is it common to the different adipokinetic agents?

4. Do patients with hormonal abnormalities such as thyrotoxicosis, myxoedema or acromegaly show any alteration from normal in the fatty acid composition of their serum lipids?

5. If so, are the changes consistent with those observed during administration of the relevant hormones?

6. Are the changes observed in patients with chronic ischaemic heart disease or in patients immediately after acute vascular occlusion comparable to those produced by endocrine influences?

Many workers have been discouraged from conducting investigations in this particular field on account of the long and laborious procedures involved. An attempt has therefore been made to develop a technique which is relatively rapid and suitable for routine use. This will be described in detail. It has been applied in order to obtain answers to some of the above questions. Greater knowledge of these problems will advance our understanding of the factors leading to disordered lipid metabolism in patients with ischaemic heart disease, as well as in those with endocrine abnormalities.

INTRODUCTION

REVIEW OF LITERATURE ON SERUM FATTY ACIDS

Historical

With the development of new techniques during the last decade, knowledge about the fatty acids of human tissues has increased. It has been known since 1907 that the fatty acids that are components of animal cells usually have an even number of carbon atoms and include saturated and unsaturated derivatives (Raper, 1907). As early as 1902, Jaecle reported the presence of palmitic, stearic and oleic acids in human fat by the application of a method based upon the different solubilities of their lead salts. The presence of fatty acids with two and four double bonds was recognised later, employing a method based upon the different solubilities of the brom derivatives (Eckstein, 1925; Wagner, 1926).

Information regarding the degree of unsaturation of human serum lipids came first from the measurement of iodine values. Wilson and Hansen (1935), using pyridine sulphate dibromide as the halogenising agent, first suggested an iodine value of 108 for the fatty acids of human serum. The presence of linoleic and arachidonic acids in human serum was first reported by Brown and Hansen (1937), who separated the polybromides of arachidonic acid and the tetrabromides of linoleic acid on the

basis of their different solubilities in ether.

Between the years 1956 and 1958 many investigators provided more detailed information by the use of spectrophotometric methods preceded by alkaline isomerisation. In this method, the polyunsaturated acids were subjected to alkaline isomerisation and were thus transformed into conjugated fatty acids. These have specific absorption bands in the ultraviolet region and could therefore be measured by spectrophotometric techniques. In this way, Evans and his coworkers (1956) were the first to show that linoleic acid is the major unsaturated acid of human serum fatty acids. Others, employing the same technique, were concerned with the relation of plasma esterified fatty acids to atherosclerosis and hyperlipidaemia (Luddy et al., 1958; Lewis, 1958; Tuna et al., 1958; Wright et al., 1959) or with the effects of experimental diets (Michaels et al., 1959; Alfin-Slater and Jordan 1960). These studies gave evidence of the degree of unsaturation but did not lead to full structural identification of the fatty acids.

Eventually, with the application of various chromatographic techniques for the separation of lipids, together with the introduction of gas-liquid chromatography for the analyses of fatty acids (James and Martin, 1952), a key was provided to the knowledge of fatty acid composition. The principle and the

application of the method have been widely described (Phillips, 1956; Keuleman, 1959; Purnell, 1962; Buchfield and Storrs, 1962; Knox, 1962). Gradually, more information has been gathered, and this will be reviewed in the following paragraphs.

The free fatty acids (FFA).

The importance of this small fraction of serum lipids (accounting for approximately 10% of total fatty acids) has been appreciated with the development of accurate methods for the measurement of serum FFA concentrations (Dole, 1956; Gordon, 1957).

Serum FFA concentrations are now known to be influenced by many factors. Increases in serum FFA levels have been reported after prolonged fasting (Dole, 1956; Recant et al., 1963), emotional stress (Bogdonoff et al., 1959), physical activity (Carlson and Pernow, 1959, 1961, Carlson et al., 1963), cigarette smoking (Kershbaum et al., 1961, 1962; Murchison and Fyfe, 1966), exposure to cold (Nutritional Rev. 1965, Glennon et al., 1967) and following the administration of several hormonal agents. Within an hour after the administration of noradrenaline or adrenaline to men, the serum FFA increased sharply (Gordon and Cherkes, 1956; Dole, 1956; Havel and Goldfien, 1959). Similar observations were made 4 hours after the injection of human growth hormone (HGH) to fasting men

(Raben and Hollenberg, 1959) and 6 hours after administration of thyroid hormone (Rich et al., 1959).

Several pathological conditions have been reported to lead to increased levels of serum FFA. Many authors have called attention to the increased fasting concentrations of serum FFA in patients with a history of myocardial infarction (Schrade et al., 1961; Kershbaum and Bellet, 1964; Rifkind, 1966). While very high levels were observed immediately after acute myocardial infarction (Kurien and Oliver, 1966), normal levels are said to be present three weeks after the acute event (Rifkind, 1966).

In view of the great interest focussed upon the levels of serum FFA and their responses to various influences, it is surprising that so little is known about the participation of individual fatty acids in increases or decreases of serum FFA concentration.

Only a few authors employing gas-liquid chromatographic techniques have described the composition of serum FFA in health (Lindgreen and Nichols, 1961; Dole et al., 1959; Schrade et al., 1961; Böttcher and Woodford, 1961) and found that oleic, palmitic and stearic acids are the main components of this fraction. The composition of serum FFA has been estimated in certain pathological conditions such as ischaemic heart disease (Schrade et al., 1961; Böttcher and Woodford, 1961) diabetes mellitus (Schrade et al., 1963), pancreatic cystic

fibrosis and in liver disease (Caren and Corbo, 1966). In patients with ischaemic heart disease, Schrade et al., (1961) observed lower proportions of polyunsaturated acids, while Bottcher and Woodford (1961) did not find any significant changes from normal, although small increases were observed in the percentages of the α-lipoprotein FFA oleic acid. Dole et al., (1959) studied the changes during alimentary hyperlipidaemia, and observed that both oleic and palmitic acids decreased in this situation together with the total FFA concentration. Soloff and Schwartz (1966) recently studied changes during the glucose tolerance test in normal and post myocardial infarct patients. The data of these authors show that the decrease in serum FFA that occurred $\frac{1}{2}$ to 3 hours after the glucose load were accompanied by an increased percentage of stearic acid and a decreased percentage of oleic acid in the total FFA fraction. More recently, Murchison and Fyfe (1966) reported increased percentages of serum FFA monounsaturated acids after cigarette smoking.

From these few publications, it is difficult to conclude whether any relationship exists between total serum FFA levels and their fatty acid composition, and no evidence is available concerning the effects of hormones or other adipokinetic agents.

The fatty acids of esterified lipids.

Dole et al., (1959) stated that "the blood plasma of a 70 kg. man contains about 50 mEq of fatty acids (approximately 25 mEq in phospholipids, 12 mEq in cholesterol esters, 10 mEq in triglycerides and 3 mEq in nonesterified fatty acids)". It is beyond the scope of this chapter to describe the conditions associated with increased concentrations of esterified lipids since these have been largely described elsewhere.

There now exists a mass of data on the fatty acid composition of serum esterified lipids in various conditions. The fatty acid composition of the individual lipid fractions represents a pattern which is fairly constant in normal physiological conditions (Hallgreen et al., 1960; Lindgreen et al., 1961; Schrade et al., 1961; Lawrie et al., 1961; Caren and Corbo, 1966; Laudat et al., 1966).

Palmitic acid is incorporated mainly into the phospholipid fatty acids, oleic acid mainly into triglyceride fatty acids, and to a lesser extent into phospholipid and cholesteryl fatty acids, while linoleic and arachidonic acids are mostly distributed between the cholesteryl and phospholipid fatty acids.

The fatty acid composition of esterified serum lipids has been shown to depend on several factors, which will be reviewed below.

Age differences

Several studies have revealed differences in the fatty acid composition of individual esterified lipid fractions in relation to age. Swell et al., (1960) have reported that children have significantly more linoleic acid in their serum cholesteryl fatty acids than do adults. Schrade et al., (1961) compared the fatty acid composition of normal males in the two age ranges 19-42 and 46-71 and found that the older group had higher percentages of saturated and monounsaturated acids and lower percentages of polyunsaturated acids in all lipid fractions.

Sex differences

Information on this matter is fragmentary. Lawrie et al., (1961) reported higher triglyceride palmitate and linoleate in women than in men. The authors, however, point out that the differences observed may in part be due to a difference of one decade between the mean ages of the groups studied. The study of Baker et al., (1966) strongly suggests that women have more palmitic and oleic acid in their total lipid fatty acids than do men.

Genetic and environmental factors

Dole et al., (1959) observed that Americans appear to have a higher content of linoleic acid in the combined cholesterol - triglyceride fraction than their British contemporaries (33.5 per cent against 12.4 per cent). The British group showed somewhat

higher levels of oleic acid in the combined fraction (34.7 per cent against 26.4 per cent). The authors assumed that these differences were due to higher cholesterol/triglyceride ratios in the American subjects. In the phospholipid fatty acids the authors observed that palmitic acid was somewhat higher and arachidonic acid lower in the American group. Scott et al., (1963) reported differences in fatty acid composition between New Yorkers and East Africans. The New Yorkers had higher percentages of cholesteryl,triglyceride and phospholipid linoleate than East Africans of both upper and lower classes. These differences were particularly interesting, because the East Africans belonging to the "upper class" did not differ from the New Yorkers in their average levels of serum cholesterol. The factors responsible for the differences have been attributed either to genetic or to environmental causes (Goodman, 1965).

Experimental diets.

Several studies, which will be cited below, have revealed that a diet rich in linoleic acid causes the relative percentage of linoleic acid in the esterified lipids to increase and the serum cholesterol concentration to fall, whereas diets rich in saturated and monounsaturated acids and in carbohydrate have an opposite effect.

Studies employing alkali isomerisation techniques and iodine value measurements provided some evidence on this matter. Michaels et al., (1959) observed that a diet rich in ethyl linoleate resulted in an increase of cholesteryl diunsaturated acids, and a decrease in saturated and monounsaturated acids. On the other hand, a diet rich in ethyl oleate or in carbohydrate resulted in a decrease in cholesteryl diunsaturated fatty acids, and an increase in saturated and monounsaturated acids. In another study it has been shown that the addition of safflower oil (rich in unsaturated acids) to the usual diet, resulted in an increase in the value of the cholesteryl fatty acid iodine number (Alfin Slater and Jordan 1960).

This information was confirmed by studies employing gas-liquid chromatographic techniques (Okey et al., 1960; Swell et al., 1962; Gunning et al., 1963). During these dietetic experiments, elevation of the serum cholesterol concentrations were found to be associated with an increase in the monounsaturated and a decrease in the diunsaturated fatty acids in the cholesterol ester fraction.

Pharmacological agents.

The effects of compounds which lower the concentration of serum cholesterol were investigated. Treatment with ethyl- α -para-chloro-phenoxyisobutyrate (Atromid S) leads to increases in monounsaturated acids and decreases in linoleic acid in

serum cholesteryl, triglyceride and phospholipid fatty acids (Jurand and Oliver, 1963). Similar effects in the cholesteryl fatty acids were reported after treatment with Atromid (Berry et al., 1963), neomycin alone and neomycin with nicotinic acid (Goldsmith, 1962). These observations are clearly in contrast to those described in the final paragraph of the section relating to the effects of experimental diets, since pharmacological agents which lower serum cholesterol affected the fatty acid pattern in the same direction as diets which led to elevation of serum cholesterol.

In patients with ischaemic heart disease the earlier reports were conflicting. James (1957), in a study of ischaemic heart disease, did not observe any significant difference in the fatty acids of the combined cholesterol and triglyceride fraction compared with normals. He suggested, however, the possibility of a small increase in oleic acid in this combined fraction. Böttcher and Woodford (1961) studied the fatty acids of lipids extracted from the protein precipitates of Cohn fraction 1-111 and of fraction 1V-V and observed differences of no statistical significance between normal and atherosclerotic patients. Other studies, carried out by Schrade et al., (1958, 1959, 1961) and by Swell et al., (1960), provided evidence that atherosclerosis, particularly if associated with hyperlipidaemia, is accompanied by a decrease in polyunsaturated acids and by an increase in

saturated and monounsaturated acids. Similarly, in diabetes mellitus, increased percentages of cholesteryl saturated and monounsaturated acids and decreased percentages of unsaturated acids were reported (Schrade et al., 1963), although, earlier, Hallgreen et al., (1961) had not found any changes in this disease. Recently, similar changes in the fatty acid composition have also been observed in multiple sclerosis (Baker et al., 1964), in familial hypercholesterolaemia (Laudat et al., 1966), and also in pancreatic cystic fibrosis and liver disease (Caren and Corbo, 1966).

Hormonal agents.

Hitherto, the influence of hormone administration or of endogenous hormonal abnormalities upon the fatty acid pattern of all four serum lipid fractions have not been described in human or animal studies. However, Boyd (1963) has studied the cholesterol ester fraction and has reported that treatment of males with D-thyroxine (3, 5, 3'5'-tetraiodo-D-thyronine) resulted in an increase in cholesteryl palmitate, palmitoleate and arachidonate and a decrease in cholesteryl linoleate. This author also observed that treatment of males with ethinyloestradiol resulted in greater decreases of cholesteryl linoleate than those induced by D-thyroxine treatment. Changes in other lipid fractions were not reported.

The above review demonstrates that most investigators apart from Boyd have studied the fatty acid composition of serum lipids only in relation to hyperlipidaemia or disease. The effects of hormones or other factors promoting an increased mobilisation of serum FFA remain largely unelucidated.

PLAN OF THE STUDY

The present study comprised investigations into changes in the composition of free fatty acids, the fatty acids of triglycerides, phospholipids and cholesterol esters in serum following mobilisation of free fatty acids. The following agents influencing the mobilisation of free fatty acids were studied.

1. Thyroid hormones

The relation between fatty acid mobilisation and composition was studied with respect to thyroid activity. For this purpose the changes which occurred after the administration of thyroid hormones to both normal and hypothyroid subjects were investigated. In addition, studies were undertaken of the changes occurring in thyrotoxic patients as a result of antithyroid therapy.

2. Human growth hormone (HGH)

Changes occurring in the fasting state as a result of daily administration of HGH were investigated. Effects of HGH were studied also hourly after HGH injection. In addition, the fatty acid pattern was studied in patients with acromegaly.

3. Noradrenaline

Changes induced during infusion of noradrenaline were studied.

4. Fasting

Effects induced by an 8-hour prolongation of a normal

overnight fast were investigated.

5. Acute and chronic ischaemic heart disease

The pattern of serum lipid fatty acids was studied within 12 hours after the onset of myocardial infarction, when the serum FFA levels are known to be very high (Kurien and Oliver, 1966). In addition the changes occurring during the next two days were followed using fasting blood samples.

The characteristics of the fatty acid composition in patients with chronic ischaemic heart disease were reinvestigated by a comparison of diseased patients with normal healthy subjects of similar age.

INVESTIGATIONS AND SUBJECTS STUDIED

Numerous investigations were made in order to study physiological influences and hormone-induced changes in serum lipids and their fatty acid composition. All the observations reported here were made in healthy subjects or in patients in the wards of the Royal Infirmary of Edinburgh.

INVESTIGATIONS ON THE EFFECTS OF THYROID HORMONES AND THYROID STATUS

(i) Thyroid hormone administration to normal humans

Daily doses of 100 µg. of 3, 5, 3'-triiodo-L-thyronine (LT_3) were administered for a period of seven days to two healthy men aged 23 and 30. For three weeks both men were kept on a diet with fixed calorie, carbohydrate, protein and fat intake. This dietetic control was begun five days prior to the control estimations and seven days prior to the administration of the hormone. Blood specimens were taken twice before, on two or three occasions during, and twice after the administration of LT_3.

(ii) Myxoedema and the response to treatment with thyroid hormones

Nine patients suffering from myxoedema (8 post-menopausal females and 1 male aged 60) were studied before and during treatment with LT_3 or L-thyroxine (LT_4). The serum lipids were studied at intervals from two weeks to eleven months.

Two of the women (R.J., and L.J.) received a controlled balanced diet during the study. In the pretreatment period they were in hospital for one week and after treatment for one week. During the period of their stay in hospital their diet consisted of a fixed calorie intake, and the proportions of carbohydrate, fat and protein remained constant. The remaining subjects were out-patients on a free diet; blood samples were always obtained at the same time after an overnight fast.

(iii) Thyrotoxicosis and the response to treatment with antithyroid therapy

Seven thyrotoxic patients were studied, two men and two women between 50 and 60 years of age and three women between 20 and 30 years of age. The effects of antithyroid therapy with carbimazole or with radioactive iodine were studied for periods of up to eleven months. In addition one thyrotoxic male aged 65 was studied in the untreated state. One of the male subjects (W.H.) was kept in hospital during the period of study, for one week prior to treatment and for a further week after attainment of the euthyroid state, and during this time strict

dietetic control was exercised. The patients received a diet of constant calorie, carbohydrate, fat and protein composition. The remaining subjects were out-patients on a free diet. Blood specimens were taken after an overnight fast at the same time each morning.

INVESTIGATIONS ON THE EFFECTS OF THE ADMINISTRATION OF HUMAN GROWTH HORMONE (HGH) AND THE EFFECTS OF ACROMEGALY

(i) Experiment designed to study long-term effects of HGH injection

The long-term effects of HGH injection were studied 24 hours after each daily injection. Four men were selected for this study. Three were between 50 and 60 years of age and suffered from ischaemic heart disease. The fourth was an 18-year old volunteer. They were in hospital for 4 weeks and were maintained during the study on a diet resembling as closely as possible their usual calorie intake, but with a constant carbohydrate and constant animal and vegetable fat composition. After a control period of one week three of the men received intramuscularly 25 mg of HGH daily for three days, and one for four days. The injections were given after an overnight fast and after the withdrawal of fasting blood samples. Additional blood samples were taken at noon and at 4 p.m.; these latter samples were analysed only for the concentrations

of serum FFA whereas the fasting samples were submitted to the full analytical procedure. Blood samples were withdrawn at the same time each morning, at noon and in the afternoon during the pretreatment period, during administration of the hormone and in the post-treatment period (3-5 days after cessation of HGH).

(ii) Experiment designed to study short and long-term effects of HGH injection

This experiment was designed to study those effects of HGH produced within a few hours of the injection and those produced after 24 hours.

Three men between 50 and 60 years were selected for the study. All three were convalescents after renal colic, 12-15 days after the event. The patients were confined to hospital for the duration of the study. Their diet was designed to approximate to their usual calorie intake, and consisted of fixed amounts of carbohydrate, fat and protein. The patients started on this diet four days before the first blood samples were taken and one week before the beginning of hormone injection. Each subject received an intramuscular injection of 25 mg of HGH on two consecutive days after an overnight fast. The first injection was followed by an 8-hour fast during which the short term effects of HGH were investigated. The second injection was followed by the usual daily meals from the balanced diet. Blood specimens taken every morning following the overnight fast were analysed for the long-term effects of the injection administered 24 hours

previously. For control purposes, blood specimens were obtained during the pretreatment period at 15, 19 and 23 hours after the commencement of fasting.

The human growth hormone used in studies (i) and (ii) was obtained from the Medical Research Council's Department of Biological Standards. Concerning the possibility of TSH contamination, the Department states: "The method of extraction is such that it is most unlikely that TSH contamination occurs; assays performed on batches prepared by the Raben method have confirmed that there is little if any (TSH activity)* detectable".

(iii) Acromegaly

Three women aged 50 - 60 were studied. The clinical diagnosis was supported in each patient by elevation of the levels of HGH to between 62 and 94 mμg/ml (Growth hormone estimations were kindly performed by Dr. W.M. Hunter in the M.R.C. Clinical Endocrinology Research Unit by the method of Hunter and Greenwood (1962). The patients under investigation were in hospital, and were kept on a normal hospital diet.

*Authors insert

INVESTIGATION ON THE EFFECTS OF NORADRENALINE

Six subjects were investigated; two were normal, two were hypertensive, and two had ischaemic heart disease. All were aged between 26 and 45 years. Fasting blood specimens from these subjects were obtained before and after the infusion of 100 µg of noradrenaline given over a period of 15 minutes.

INVESTIGATION ON THE EFFECTS OF FASTING

The effects of prolongation of an overnight fast by a further 8 hours were studied in 12 subjects, 3 healthy volunteers and 9 hospital patients. The period of fast started after the evening meal at 6 p.m. and lasted until 5 p.m. on the next day. All subjects were resting during the period of fast. Free access to water was allowed during the fasting period. The hospital patients were subjects with ischaemic heart disease, and those convalescent following renal colic.

INVESTIGATIONS ON THE EFFECTS OF ISCHAEMIC HEART DISEASE (IHD)

(1) Acute myocardial infarction

A study was carried out on 8 patients admitted to the Coronary Care Unit of the Royal Infirmary, Edinburgh within 12 hours of the onset of pain. All had electrocardiographic signs of myocardial infarction according to the W.H.O. criteria (World Health Organisation, 1959) together with a rise in serum creatine kinase

(Smith, 1967) and glutamic-oxaloacetic-transaminase (Reitman and Frankel, 1957). In 7 of 9 patients, estimations were carried out on the day of admission and on two consecutive subsequent days, the second and third samples being taken after an overnight fast. The remaining two patients had samples taken only on the day of admission and on the next day, after an overnight fast.

(ii) Patients with angina or post-acute myocardial infarction

In all, 23 men aged 33-51 were examined; 13 had angina, and 10 had had a myocardial infarction in the past (more than two months prior to the study). All the subjects were on a free diet. Blood specimens were taken after an overnight fast at the same time each morning.

(iii) Control groups

The control group for the above study,(ii), comprised 12 healthy men aged 26 - 51 years, the average age in this control group being 3 years less than in the group with ischaemic heart disease (33 against 36).

The control group for the studies of hormonal influences comprised four healthy post-menopausal women aged 50-60 years.

GENERAL COMMENTS ON THE EXPERIMENTAL CONDITIONS AND ON THE CHOICE OF PATIENTS

Clinical investigations should be undertaken in individuals who are sufficiently similar that they can be grouped into one category e.g., they should all be healthy or all have the same disease or disorder. This principle could not always be observed in the present work; for example, the studies whose aim was to elucidate the effects of fasting, of growth hormone and of noradrenaline have some weaknesses. The subjects investigated consisted of healthy members of staff, patients convalescing after renal colic, hypertensive subjects and subjects with ischaemic heart disease.

There were difficulties in the standardisation of conditions for the growth hormone study. Only one healthy 18-year old male volunteer agreed to stay in hospital for 4 weeks under controlled dietary conditions for the purpose of the growth hormone study. It is most probable that if this experiment had been carried out on two groups of subjects, one healthy and one with ischaemic heart disease, some differences would have been noted, which could not be elucidated in this limited study. The same suggestions apply to the studies on the effects of fasting and on the effects of noradrenaline.

The studies on the effects of thyroid hormones were performed in more homogeneous groups and the results can be

regarded as more conclusive. It was not always possible to observe strict conditions of dietary control in all subjects.

METHODS

CONSIDERATION OF ANALYTICAL TECHNIQUES

When this study was begun in January 1963, the gas-liquid chromatographic analyses of fatty acids in the four main lipid fractions in a single specimen of serum required fourteen days (Böttcher et al., 1959). Only very slow progress can be expected with such a time-consuming technique. The procedure consists of many steps, including preparation of the total lipid extract, separation of lipids into chemical classes, isolation of fatty acids from their esters by means of hydrolysis, the removal of fatty acids from their soaps by acidification and re-extraction, the methylation of fatty acids and the gas-liquid chromatographic analysis of the resultant fatty acid methyl esters.

The separation of lipids into each class is the most time-consuming step in the preparatory techniques since column chromatography is required. The more rapid technique of thin-layer chromatography has the disadvantage that the amounts of lipid separated are quantitatively insufficient for further analyses. In addition, losses of polyunsaturated acids during chromatography on thin-layer plates have been reported (Nichanan et al., 1963; Malins and Mangold, 1960; Imaichi et al., 1966).

The present study is concerned with the problem of separation of lipids by column chromatography, employing a system of two small rapidly running columns. The purpose of the first column is to separate phospholipids from the remainder of the lipids, and that of the second column is to separate cholesterol esters, triglyceride esters and free fatty acids.

For the separation of phospholipids from the rest of the lipids the original method of Borgström (1952) was re-investigated. In this method a silicic acid column is used as a selective filter which retains phospholipids, while neutral lipids and free fatty acids are eluted from the column with chloroform. The use of a silicic acid column for the separation of phospholipids from the rest of the lipids has been criticised on several grounds. Artifact formation has been observed during lipid chromatography on silicic acid (Olley, 1956; Collins, 1960). Some phospholipids may be irreversibly adsorbed (Luddy et al., 1958) or, alternatively, prematurely eluted (Lipsky et al., 1957). In addition, the separation requires a large volume of solvent for the elution of adsorbed phospholipids (Bottcher, personal communication).

In the techniques described, silicic acid was dehydrated either by oven heating (Borgström, 1952) or by pre-washing with dehydrating solvents (Hirsh and Ahrens, 1958) in order to increase its adsorptive strength (Trueblood and Malmberg, 1954).

However, the use of silicic acid in a highly activated state has been reported to result in reduced reproducibility of chromatograms, due to increased activity of "side-effects" such as irreversible adsorption and catalytic transformations of lipid molecules (Böttcher et al., 1959).

The present study attempted to overcome these weaknesses in the method by the application of silicic acid of a higher degree of hydration and of a coarser mesh. For this purpose a study was made of the effects of hydration of the silica gel (mesh 60-100) on the accuracy of separation, of the adsorption capacity and of the hazard of "side-effects" on the fatty acid components.

For the separation of the remaining lipid fraction, comprising cholesterol esters, triglyceride esters and free fatty acids, column chromatography using Florisil (synthetic magnesium silicate) has been investigated. This adsorbent was chosen since Carrol (1960) has shown that palmitic acid esters of different lipid classes can be separated successfully with Florisil and the procedure requires only one-sixth of the time and one-third of the solvents necessary for the separation of the same quantity of lipids from a similar amount of silicic acid. This method had already been proposed for the separation of serum lipids (Carrol, 1960) but it had not

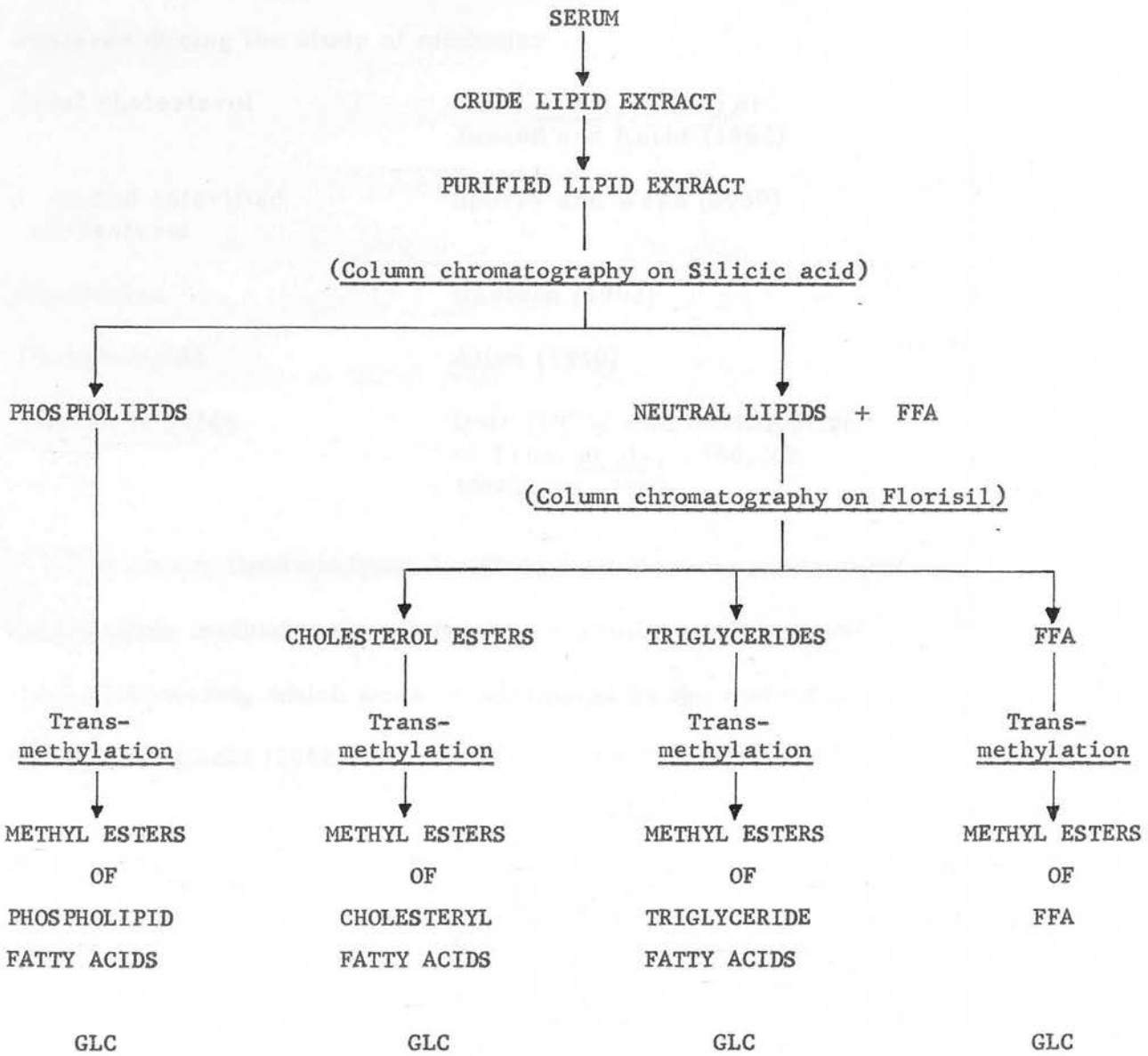
previously been studied in detail for this purpose. Neither was completeness of the separation demonstrated, nor were the recoveries of the relevant fractions determined. The existence of any selective effect upon the structure of individual fatty acids during passage through the column was also not determined. The chromatographic properties of Florisil in respect of serum lipids were studied here in order to determine the optimal conditions for the separation of triglycerides, cholesterol esters and free fatty acids for the purpose of the gas-liquid chromatographic analyses of fatty acid components of the relevant lipids.

Apart from the separation techniques, the other steps in the procedure, such as preparation of total lipid extract, preparation of fatty acid methyl esters and gas-liquid chromatographic analysis were adopted from the methods recommended by other authors as described in the following paragraphs.

The adopted sequence of the procedure is demonstrated on the following diagram.

L A Y - O U T

O F T H E P R O C E D U R E



Abbreviations: GLC - Gas-liquid chromatography.
FFA - Free fatty acids

METHODS USED FOR THE DETERMINATION OF SERUM LIPIDS

The following colorimetric methods were used for the lipid analyses during the study of methods:

Total cholesterol	Abell et al., (1952) or Jurand and Recht (1962)
Free and esterified cholesterol	Sperry and Webb (1950)
Glycerides	Carlson (1963)
Phospholipids	Allen (1940)
Free fatty acids	Dole (1956) with modification of Trout et al., 1960, or Mosinger, 1965

The serum lipid analyses in clinical studies were determined by the same methods, except for the determination of serum total cholesterol, which were all estimated by the method of Jurand and Recht (1962).

GENERAL RULES FOLLOWED IN THE ANALYTICAL PROCEDURE

The following rules in all analytical procedures were adhered to:

1. Serum lipid extracts were prepared within one hour after withdrawal.
2. Extracts of lipids in all analytical stages were stored at -10°C.
3. Evaporation of solvents from lipid extracts was carried out under nitrogen.
4. All solvents were Analytical Grade. Hexane and petroleum ether were free from aromatic compounds. Except for diethyl ether, peroxide free, all solvents were re-distilled from glass prior to use.

PREPARATION OF TOTAL LIPID EXTRACT

The crude lipid extract was prepared according to the recommendations of Folch et al., (1957) using for one volume of serum 25 volumes of chloroform methanol 2:1 (v/v). (Authors recommended the use of 1 volume of serum and 17-25 volumes of solvent mixture). The lipid extract was refluxed for 30 minutes at 63°, since Böttcher et al., (1959) have shown that refluxing is essential to obtain higher than 98-99% yield of lipids.

The non-lipid components were removed by the technique of Folch et al., (1957) with the difference that the volume of saline employed was 16 per cent instead of 20 per cent of the volume of extract. This was done on the basis that the serum itself contributes 4 per cent of the water phase, (the authors' experience was derived from work with dry tissue). In fact, other authors have found that, in the case of serum lipid extract, the reduction of the water phase to as low as 10 per cent, gave a more efficient extract (Iongh&Van Pelt, 1962) than the use of 20 per cent of water phase as proposed by Folch et al., (1957).

THE SEPARATION OF PHOSPHOLIPIDS FROM THE REST OF THE SERUM LIPIDS

(i) Description of the procedure finally adopted

Silica Gel "Davison 950" mesh 60-100 was used. Portions containing a few grams of silicic acid were roasted on a mild flame for 10 minutes, and cooled for 10 minutes. 4cc of adsorbent were poured into 20 ml of chloroform containing 0.2 ml of water. The slurry was mixed for a few minutes and columns measuring 7 mm in diameter and 17 cm in length were packed with the slurry.

2 ml of the total lipid extract in chloroform were applied to the top of the column. Neutral lipids and free fatty acids (FFA) were eluted from the column with 50 ml of chloroform. The column was then washed with 10 ml of acetone and the phospholipids eluted with 20 ml of methanol-chloroform 4:1 (v/v), followed by 100 ml of methanol.

The above conditions were selected on the basis of the study described below.

(ii) Description of the study

Preconditioning of silica gel

Silica Gel "Davison 950" mesh 60-100 was employed in the three following ways: (1) Adsorbent was dried by roasting on a mild flame for 10 minutes, and cooled in contact with air, using a standardised time of 10 minutes. (2) Adsorbent was dried by heating in an oven at 120° for 24 hours and cooled in contact with air using a standardised time of 10 minutes. (3) Adsorbent was prepared as in (1) and was hydrated with 8% of water as follows: a volume of 4 cc (corresponding to a weight of 2.5 g of silicic acid) was poured into approximately 20 ml of chloroform containing 0.2 ml of water (8% by weight). The slurry of silicic acid with chloroform was stirred for one minute before the columns were packed.

It appeared that the procedure of roasting did not destroy the gel, since its physical properties in relation to its capacity to adsorb water after the roasting procedure were in no way different from those observed after oven drying. Roasting was therefore preferred, since it reduced the risk of accidental adsorption of unidentified materials in the vapour phase in the atmosphere within the oven.

Columns for chromatography

The columns employed measured 0.7 cm in diameter and 17 cm in length, were constricted at their lower extremity and had an expanded solvent reservoir to hold 50 ml. They were packed with the prepared slurry of silicic acid in chloroform and the walls of the column were washed down several times with small portions of chloroform. The columns were neither temperature controlled nor run under pressure.

Solvent system

The total lipid extract was applied in 2 ml of chloroform. The neutral lipids were eluted with 50 ml of chloroform, and the phospholipids with 20 ml of methanol-chloroform 4:1, and subsequently with 100 ml of pure methanol. Between the first and second fractions the columns were washed with 10 ml of acetone. (This acetone wash was recommended by Nelson (1962) for the removal of products of oxidation from the columns).

Elution curves of serum lipids from columns containing dried silicic acid and from columns containing silicic acid hydrated to 8%

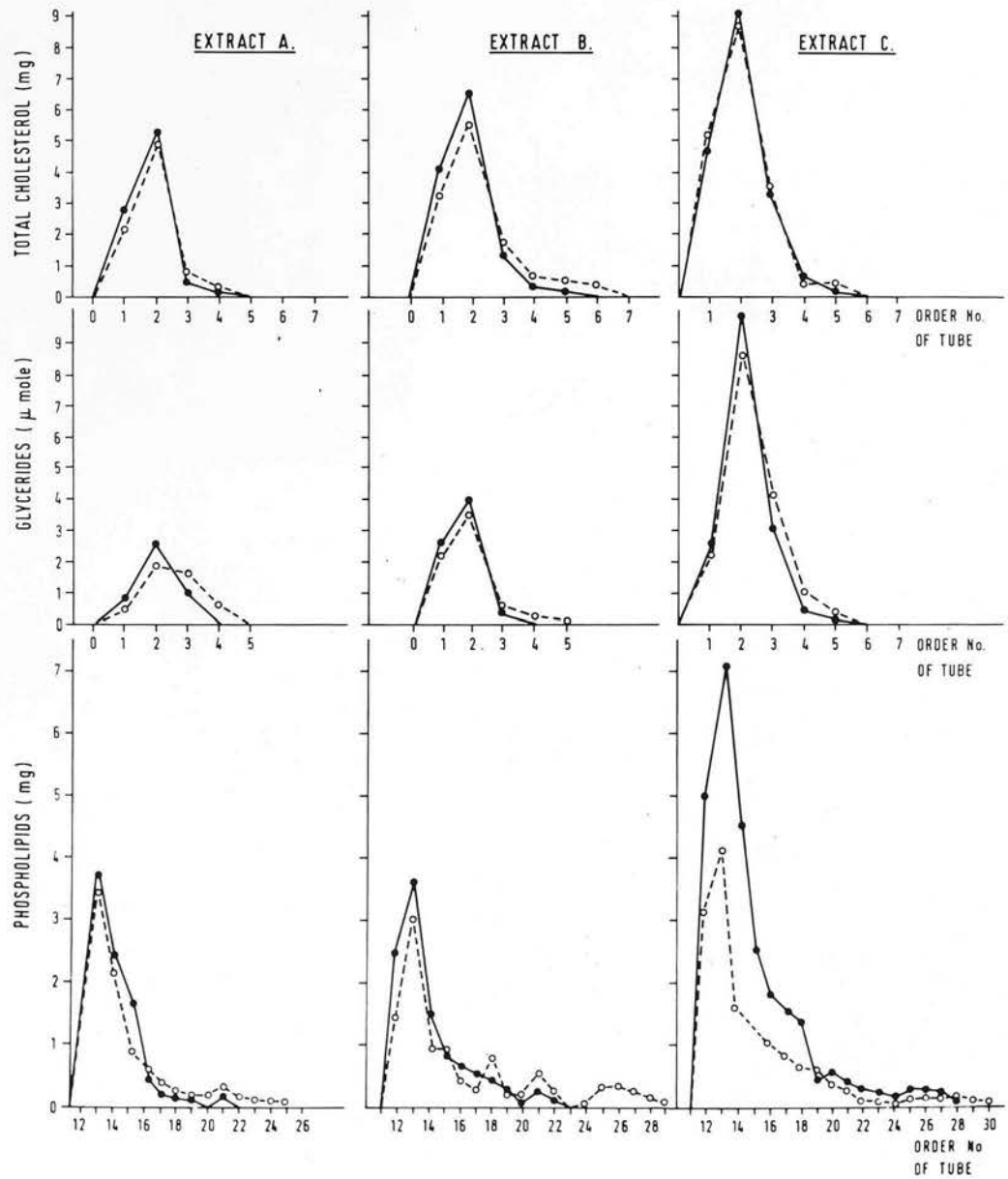
Fig. 1 illustrates the pattern of the elution of serum lipids (cholesterol containing compounds, glycerides and phospholipids) from 2.5 g. silicic acid columns, which in one case contained silicic acid dried by mild roasting, and in the second, silicic acid hydrated to 8%. Hydration has a negligible effect on elution curves of total cholesterol (esters and free) and glycerides, but influences the elution curves of phospholipids.

Fig. 1 Elution curves of serum lipids from columns containing dried silicic acid and silicic acid hydrated to 8%

Extracts A, B and C represent the total serum lipids from 3 ml of normal serum, 3 ml of hyperlipidaemic serum and 6 ml of hyperlipidaemic serum respectively, each dissolved in 2 ml of chloroform. Each of these extracts was applied to two columns (0.7 x 17.0 cm), one containing silicic acid dried by roasting, and the other, silicic acid hydrated to 8%. The elution curves of cholesterol and glycerides are illustrated separately, although they emerge from the column together. The solvent system applied was chloroform (50 ml) for elution of neutral lipids (and FFA), acetone (10 ml) for washing out the column, and methanol-chloroform 4:1 (20 ml) followed by methanol (140 ml). Each tube collected contained 5 ml of eluant. The lipids were determined by the following methods:
total cholesterol - Abell et al., 1952, glycerides (as triglycerides) - Carlson, 1963, and phospholipids Allen, 1940.

•—• column with hydrated silicic acid

o---o columns with dried silicic acid


EXTRACT A.
EXTRACT B.
EXTRACT C.
TOTAL CHOLESTEROL (mg)
GLYCERIDES (μ mole)
PHOSPHOLIPIDS (mg)
ORDER No. OF TUBE
ORDER No. OF TUBE
ORDER No OF TUBE

These differences in the elution curves of phospholipids from those columns containing dried silicic acid and from those containing hydrated silicic acid occur despite the fact that, in theory at least, the acetone wash employed before phospholipid elution should have effectively "dehydrated" the silicic acid. With the use of the dried silicic acid, only 60-65% of phospholipids emerge with the first 30 ml of methanol-containing solvents. With the use of silicic acid hydrated to 8%, 85-90% of phospholipids emerge with the same volume of solvent. Moreover, the tailing of the phospholipid elution was slightly reduced by the use of the hydrated silicic acid which has been described.

Adsorption capacity for serum phospholipids of silicic acid dried or hydrated to 8% during column chromatography

The columns containing 2.5 g of silicic acid dried either by roasting or by oven-heat were not capable of adsorbing amounts of phospholipids exceeding 11-12 mg. When the load of phospholipids was greater, premature elution of phospholipids together with the fraction of the neutral lipids took place (Table 1). On columns containing 2.5 g of silicic acid hydrated to 8%, amounts of phospholipids up to 23.8 mg were successfully adsorbed without premature elution. Thus, the silicic acid hydrated to 8% had an adsorption capacity for the serum

TABLE 1.

Recoveries of serum phospholipids from 2.5g silicic acid column with different contents of adsorbed ("free") water

Method of preparation of silica gel	Approx. content of adsorbed water	Charge on column* mg.	Amount recovered			Percent of Recovery in Eluant No. 3
			Eluant No. 1 mg.	Eluant No. 2 mg.	Eluant No. 3 mg.	
Oven-dried (at 120°C for 24 hrs)	2 - 2.5%	8.90	-	-	8.60	97
		13.62	2.70	-	11.20	82
		15.50	4.00	0.58	11.58	75
		20.50	7.79	0.80	11.90	58
Dried by mild roasting for 10 minutes	1 - 1.2%	4.78	-	-	4.89	102
		9.78	-	-	9.78	100
		11.48	-	-	11.79	103
		23.12	9.20	2.10	12.64	54
		26.90	9.90	4.00	12.10	45
Untreated	4.5 - 5.0%	20.50	7.00	0.20	13.30	65
Hydrated to 8% after being dried by mild roasting	8.5 - 9.5%	4.78	-	-	4.80	100
		9.78	-	-	10.00	102
		11.48	-	-	12.10	105
		23.12	-	-	23.83	103
		26.10	1.55	1.00	23.17	89

* mg of phospholipids in total serum lipid extract

Eluant No. 1 50 ml. of chloroform
Eluant No. 2 10 ml. of acetone
Eluant No. 3 20 ml. of 4:1 methanol/chloroform + 100 ml. of methanol.

phospholipids under the described conditions approximately twice as great as that of the silicic acid dried in the oven or by mild roasting. Untreated silicic acid had approximately the same adsorptive capacity as the dried material (Table 1). This represents another advantage of the hydrated silicic acid.

The recoveries of lipids from columns containing hydrated silicic acid

Columns containing 2.5 g of silicic acid hydrated to 8% were capable of separating the serum neutral lipids from phospholipids with satisfactory recoveries (Tables 1 and 2) within the tested ranges of the quantities of lipids.

Changes in the fatty acid composition of lipids occurring during the column chromatography on the dried and on the hydrated silicic acid

The results of the comparative runs of the known mixtures of FFA and cholesteryl fatty acids through columns containing silicic acid dried by mild roasting or silicic acid hydrated to 8% are presented in Table 3. Hydrated silicic acid does not appreciably change the composition of fatty acids, whereas the dried silicic acid does change that composition. In addition, the losses of the individual acids on the dried silicic acid were inconsistent.

When studies of serum lipids were undertaken on both types of adsorbent the differences were less marked than those noted

TABLE 2.

Recoveries of serum neutral lipids and free fatty acids from columns containing 2.5 g silicic acid hydrated to 8%.

Kind of lipids	Charge on column* mg.	Amount recovered mg.	Percent of recovery	Method used for estimation
Cholesterol-containing lipids	Total 4.37	4.15	95	
	Ester 2.97	2.79	94	
	Free 1.40	1.39	99	
	Total 8.50	8.25	97	Sperry and Webb (1950)
	Ester 6.38	6.17	97	
	Free 2.12	2.20	104	
	Total 13.50	13.00	96	
	Ester 10.40	9.60	96	
	Free 3.10	3.40	110	
	Total 12.00	12.20	102	
	Total 15.12	15.70	104	Jurand and Recht (1962)
	Total 19.90	18.90	95	
Triglycerides	μ moles	μmoles		
	2.74	2.75	100	
	10.00	9.50	95	
	10.60	9.96	94	
	3.89	3.78	97	Carlson (1963)
	4.20	4.05	96	
	16.70	15.90	95	
Free fatty acids (FFA)	μEq.	μ Eq		
	2.37	2.34	99	
	1.13	1.10	97	Dole (1956)
	2.62	2.55	97	
	2.43	2.01	83	
	3.13	2.94	94	
	2.52	2.84	113	Mosinger (1965)
	1.31	1.25	95	
	3.09	2.91	94	

* mg of lipids in total serum lipid extract.

TABLE 3.

The effect of hydration of silicic acid on changes in the percentage composition of free and cholesteryl fatty acids resulting from passage through the column.

A-columns packed with silicic acid hydrated to 8%, B-columns packed with silicic acid dried by roasting. Charge on column: standard mixtures of cholesterol esters, standard mixtures of free fatty acids (in separated runs).

Lipids	Charge on column mg.	Fatty acid short hand design*	Percentage composition Before chromatography	After chromatography A	After chromatography B
CHOLESTEROL ESTERS					
Mixture 1.	9	18:0	45	48	52
		18:2	39	38	37
		20:4	16	14	12
Mixture 2.	6.5	18:0	60	61	60
		18:2	26	25	29
		20:4	14	14	11
Mixture 3.	7.0	18:0	52	52	53
		18:2	48	48	47
Mixture 4.	Approx. 12	18:0	62	64	59
		20:4	37	36	41

Free fatty acids (FFA)	μg. (i) - 0.5 μg. (ii) - 1.2 μg.		A (i)	A (ii)	B (i)	B (ii)
Mixture 1.		16:0	18	19	13	15
		16:1	5	5	3	3
		18:0	5	5	7	7
		18:1	23	23	24	24
		18:2	43	41	44	40
		20:4	7	6	8	10

* 16:0 n-Hexadecanoic
16:1 cis-Δ^{9}Hexadecenoic
18:0 n-Octadecanoic
18:1 cis-Δ^{9}Octadecenoic
18:2 cis-$\Delta^{9,11}$Octadecadienoic
20:4 $\Delta^{5,8,11,14}$ Eicosatetraenoic

above for the standard mixtures. However, in two of the three sera tested, lower percentages of free palmitoleic acid were observed, and in one, lower percentages of triglyceride palmitoleate were observed in the eluate from the dried silicic acid, which may be attributed to a "side-effect" of the activated adsorbent (Table 4).

This study thus shows a further advantage of hydrated silicic acid.

Conclusions from the studies on separation of serum phospholipids by silicic acid chromatography

In summary, the use of silicic acid hydrated to 8% according to the technique described has the following advantages when compared to dried silicic acid:

(1) the adsorptive capacity is increased by a factor of two

(2) the "tailing" phenomenon observed with phospholipid elution is somewhat less marked, thus reducing the risk of loss of material

(3) the hazards of molecular destruction, or rearrangement, and of irreversible adsorption are diminished.

TABLE 4.

Effect of hydration of the silicic acid used for column chromatographic separation of serum phospholipids on the percentage composition of fatty acids in all four lipid fractions.

A-Columns packed with silicic acid hydrated to 8%.
B-Columns packed with silicic acid dried by roasting.
Charge on columns:lipid extract from 3 ml. of normal serum (Serum No. 1 and No. 2) and 3 ml. of hyperlipidaemic serum (Serum No. 3 and No. 4). The cholesterol esters, triglycerides and FFA were separated on Florisil columns according to the conditions described in the procedure finally adopted.

Phospholipid fatty acid

Fatty acid short hand design	Serum No. 1		Serum No. 2		Serum No. 3		Serum No. 4	
	A	B	A	B	A	B	A	B
16:0	23	23	26	26	33	33	31	32
16:1	2	2	1	1	2	2	2	2
18:0	19	19	15	15	17	17	17	17
18:1	21	19	14	15	15	16	12	11
18:2	21	21	32	30	23	22	23	26
20:0	1	1	Tr	Tr	Tr	Tr	Tr	Tr
20:4	9	10	8	9	9	9	11	10

Cholesteryl fatty acids

	Serum No. 1		Serum No. 2		Serum No. 3	
	A	B	A	B	A	B
16:0	7	8	10	10	9	9
16:1	3	3	3	4	3	4
18:0	1	1	Tr	1	1	1
18:1	16	17	22	24	17	15
18:2	68	66	59	57	62	62
20:4	6	5	6	5	8	9

Triglyceride fatty acids

	Serum No. 1		Serum No. 2		Serum No. 3	
	A	B	A	B	A	B
16:0	25	26	24	23	28	27
16:1	5	7	6	6	4	4
18:0	5	5	3	3	3	2
18:1	41	43	51	50	40	42
18:2	23	19	15	16	23	23

Free fatty acids (FFA)

	Serum No. 1		Serum No. 2		Serum No. 3	
	A	B	A	B	A	B
16:0	30	30	19	18	33	34
16:1	2	3	2	4	5	8
18:0	20	20	22	20	15	12
18:1	30	32	42	41	34	34
18:2	14	12	13	15	14	12
20:4	4	3	2	2	1	1

SEPARATION OF CHOLESTEROL ESTERS, TRIGLYCERIDES AND FREE FATTY ACIDS

(i) Description of the procedure finally adopted.

The phospholipid free misture of serum lipids (after passage through the silicic acid column) dissolved in chloroform was dried under nitrogen at 60°C, re-dissolved in 2 ml of hexane and applied to the top of the Florisil column. A column measuring 0.7 x 17 cm was packed with 4 g of Florisil of mesh about 100 and hydrated to 8% (see next paragraph in the section entitled "Preparation of Florisil). The fractions of separated lipids were collected simultaneously with the changing of solvents applied to the columns. The solvents applied were as follows:

Solvent	Volume (ml)	Fraction eluted
Hexane	50	hydrocarbons (discarded)
4% ether in hexane	50	Cholesterol esters (collected)
15% ether in hexane	50	Triglycerides (collected)
20% ether in hexane	20	"
Ether	20	Diglycerides (discarded)
Acetone	20	Some organic compounds probably products of the oxidation of lipids (discarded)
4% acetic acid in ether	50	Free fatty acids (collected)

(ii) Description of the study

Preparation of Florisil

Florisil, a product of the Floridin Company (mesh 60-100) activation temperature 649 °C) was employed in this study. To select the optimal conditions for lipid chromatography the following fractions of adsorbent were tested:

(1) Adsorbent mesh 60-100 (total fraction)

(2) Adsorbent mesh about 100 (obtained by rejecting that portion of the material which did not pass through a sieve of 100 mesh.

The adsorbent was used in dried or in hydrated form. Drying of the adsorbent was carried out by mild roasting for 10 minutes, followed by cooling for a standardised time of 10 minutes in contact with air. Hydration of the adsorbent was carried out after the drying procedure by a technique analogous to that described for silicic acid, the only difference being that the water required for hydration was added not to chloroform but to the hexane in which the slurry was prepared. The quantities of Florisil were measured in calibrated tubes (a volume of 8 cc corresponded to 4 g of adsorbent).

Chromatographic procedures

Two sizes of columns were tested. Large columns measuring 1.5 gm x 35 cm and small columns measuring 0.7 x 17 cm. The shape of the column was the same as that described for the separation of phospholipids.

The dry residue of serum lipids (previously freed from phospholipids by passage through a silicic acid column) was applied to the top of the Florisil columns in 2 ml of hexane. As proposed by Carrol (1961), the ether-hexane solvent system was employed for elution of lipids, and the hydrocarbons were removed with hexane. The proportions of ether in hexane required for elution of a particular lipid fraction were dependent upon the degree of hydration of the Florisil, while the required volumes of these solvents were dependent upon the amount of adsorbent used.

Selection of the optimal degree of hydration of Florisil for separation of serum cholesterol esters from triglycerides

Florisil mesh 60-100 was used for the study. Untreated Florisil, when used from a freshly opened bottle, gave satisfactory separation. However, a few months after the bottle had been in use, the characteristics of the elution diagram changed entirely (Fig. 2).

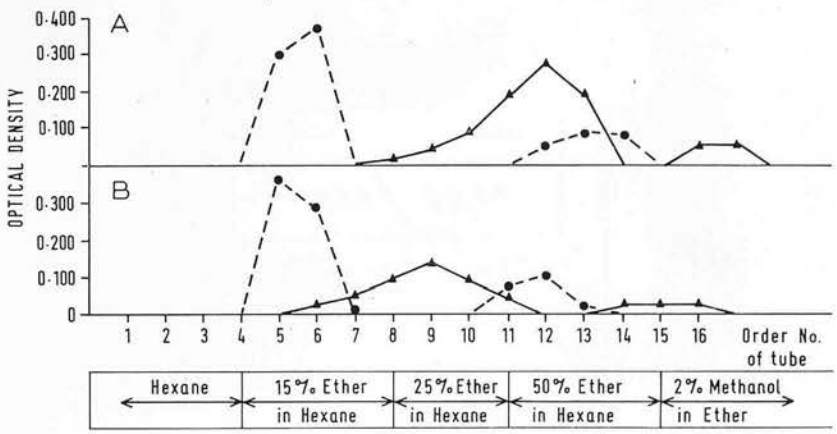
Elution diagrams for serum lipids were drawn for columns packed with Florisil hydrated to 7 or 10%, and with Florisil dried by roasting (see the paragraph entitled "Preparation of Florisil"), to select optimal conditions for the separation of cholesterol esters from triglycerides (Fig. 3). The presence of free cholesterol in the triglyceride fraction did not interfere with the estimation of triglyceride fatty acids, therefore

Fig. 2 Separation of serum lipids on columns containing untreated Florisil

A and B represent the elution diagrams of lipids from 5 ml of serum (phospholipid free) during chromatography on columns (1.5 x 35.0 cm) packed with 12 g of untreated Florisil. The A column contained Florisil from a freshly opened bottle, the B column contained Florisil from the bottle which had been in use for several months. Each tube collected contained 25 ml of eluant. The optical densities of colorimetric reactions were determined for cholesterol by the method of Sperry and Webb (1950), and for glycerides by the method of Carlson (1963).

•---• Cholesterol (ester, 1st peak; free, 2nd peak)

▲——▲ glycerides (triglycerides, 1st peak; partial glycerides, 2nd peak).


OPTICAL DENSITY
0·400
0·300
0·200
0·100
A
0·300
0·200
0·100
0
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Order No. of tube
Hexane
15% Ether in Hexane
25% Ether in Hexane
50% Ether in Hexane
2% Methanol in Ether

complete separation of cholesterol from the triglyceride fraction was not essential.

Fig. 3 demonstrates that, with increased degree of hydration, lipids are eluted with solvents of lower polarity. On columns containing dried Florisil, cholesterol esters are eluted with 15% ether in hexane, while on columns containing Florisil hydrated to 7% or 10%, cholesterol esters are eluted with 5% ether in hexane. The same observation has been made by Carrol (1961) on diagrams obtained with the mixtures of different esters of palmitic acid. The only difference between the diagrams which Carrol obtained and those presented here is that, in the case of serum lipids, the dried Florisil does not separate the cholesterol esters from the triglycerides, whereas in the case of the mixture of palmitic acid esters, used by Carrol, separation was complete. The columns packed with dried Florisil caused a broad tailing peak of cholesterol esters which overlapped the sharp peak of triglycerides. Higher degrees of hydration (10%) resulted in a sharp peak of elution of cholesterol esters, with an overlap between the cholesterol and triglycerides caused by too rapid elution of triglycerides. The optimal conditions for the separation of cholesterol esters from triglycerides were obtained with Florisil hydrated to 7%.

Selection of the amount of adsorbent and size of the column

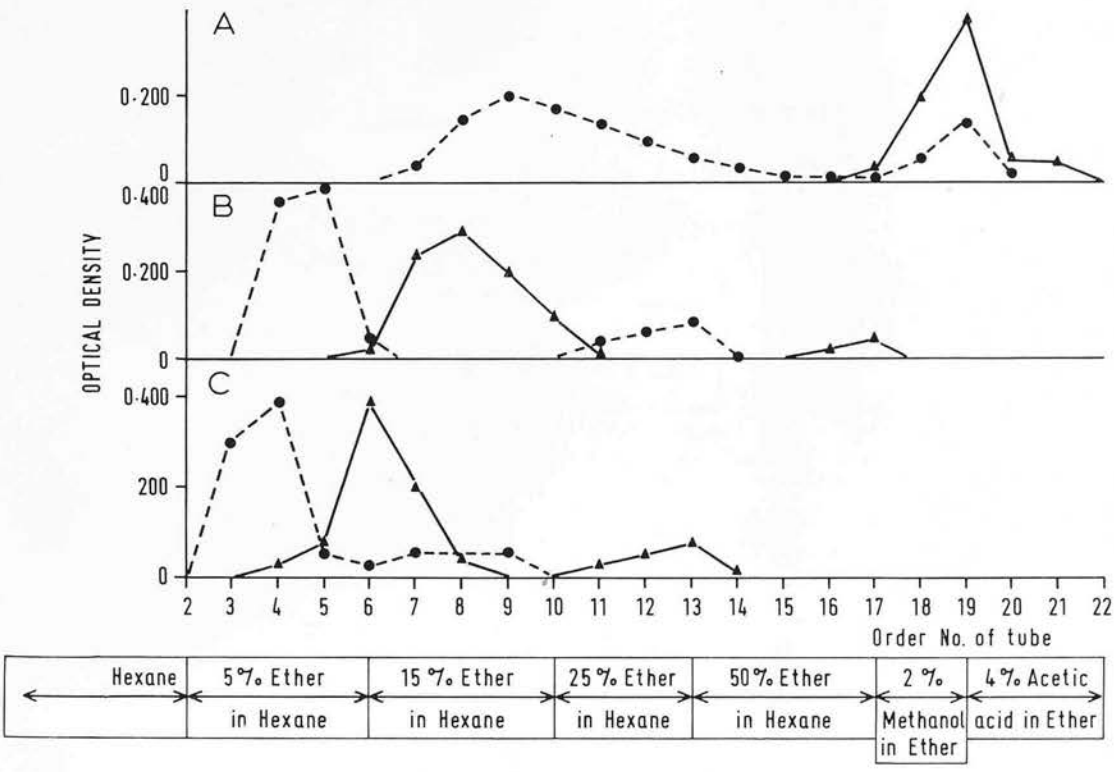
The separation of serum lipids on the 12 g columns illustrated in Figs. 2 and 3 were carried out with 500 ml of

Fig. 3 Separation of serum lipids on columns containing Florisil of different degrees of hydration

A, B and C represent elution diagrams of lipids from 5 ml of serum (phospholipid-free) during chromatography on columns (1.5 x 35.0 cm) packed with 12 g of Florisil. A — column with Florisil dried by roasting for 10 minutes, B — column with Florisil hydrated to 7%, C — column with Florisil hydrated to 10%. Each tube collected contained 25 ml of eluant. The optical densities of the colorimetric reactions were determined as shown in Fig. 2.

●---● cholesterol (ester, 1st peak; free, 2nd peak)

▲——▲ glycerides (triglycerides, 1st peak; partial glycerides, 2nd peak)


A
B
C
0·200
0
0·400
0·200
0
0·400
200
0
OPTICAL DENSITY
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Order No. of tube
Hexane
5 % Ether in Hexane
15 % Ether in Hexane
25 % Ether in Hexane
50 % Ether in Hexane
2 % Methanol in Ether
4 % Acetic acid in Ether

solvents and required 4 hours for operation; therefore, further investigations were directed to an attempt to decrease the time required and the amount of solvents used. This was achieved by the use of shorter, narrower columns containing 4 g of Florisil. Fig. 4 demonstrates the separation diagram obtained when equal aliquots of serum lipids were applied to the two types of column, a large one containing 12 g of Florisil, and a small one containing 4 g of Florisil. In both cases the adsorbent was hydrated to 8%. It has been shown that, on the smaller columns, separation could be achieved with smaller volumes of solvent, without an overlap between the relevant fractions. In addition, the duration of the procedure could be reduced from 4 hours to 75 minutes.

Selection of size of mesh

The effectiveness of separation was studied by the application of three varying quantities of lipids to each pair of columns (0.7 x 17 cm), one of which was packed with Florisil mesh 60- 100 and the other with Florisil mesh 100. As demonstrated in Fig. 5, the columns with more finely divided adsorbent gave sharper elution curves, as would be expected from basic chromatographic principles. However, it appears that the elution diagram of triglycerides is more affected by the size of

Fig. 4 Separation of serum lipids on columns differing in size and in the amount of Florisil used for packing

A and B represent elution diagrams of lipids from 3 ml of serum (phospholipid-free) during chromatography on columns containing Florisil hydrated to 8%. A — large column (1.5 x 35.0 cm) packed with 12 g of Florisil, B — small columns (0.7 x 17 cm) packed with 4 g Florisil. Each tube collected contained 10 ml of solvent. The optical densities were determined as shown in Fig. 2.

●---● cholesterol (ester, 1st peak; free, 2nd peak).

▲——▲ glycerides (triglycerides, 1st peak; partial glycerides, 2nd peak).

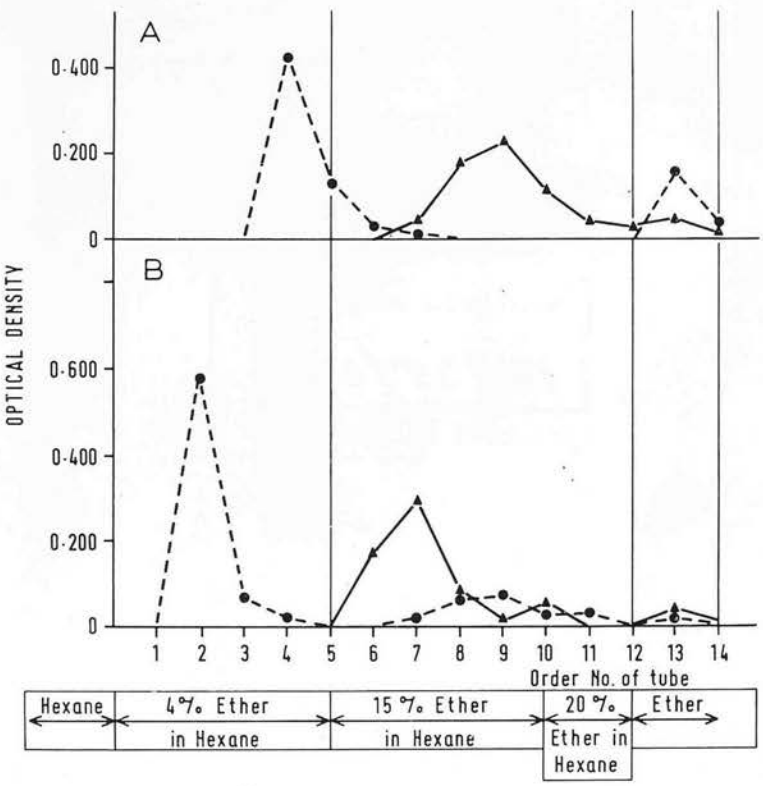

A
B
0·400
0·200
0
0·600
0·400
0·200
0
OPTICAL DENSITY
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Order No. of tube
Hexane
4% Ether in Hexane
15 % Ether in Hexane
20 % Ether in Hexane
Ether

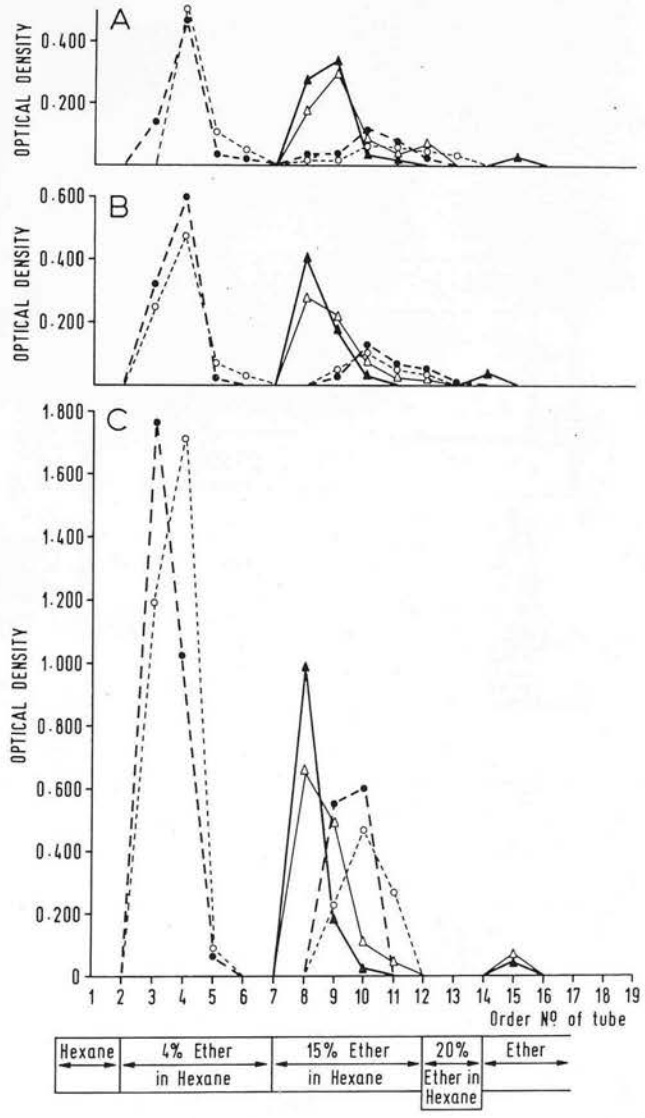
Fig. 5 Separation of lipids on columns containing Florisil of different mesh sizes

A, B and C represent elution diagrams of lipids (phospholipid-free) from 3 ml, 5 ml and 12 ml of serum respectively, during chromatography on small columns packed with 4 g of Florisil differing in mesh size.

●---● ▲——▲ columns with Florisil mesh 60 - 100

○---○ △——△ columns with Florisil mesh 100

The broken line is the elution curve of cholesterol-containing compounds and the unbroken line is the elution curve of glycerides. Each tube collected contained 10 ml. of eluant. The optical densities were determined according to the methods described in Fig. 2.


A
B
C
OPTICAL DENSITY
0·400
0·200
0·600
1·800
1·600
1·400
1·200
1·000
0·800
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Order Nº of tube
Hexane
4% Ether in Hexane
15% Ether in Hexane
20% Ether in Hexane
Ether

mesh than is that of cholesterol esters. There is a considerably greater tailing of the triglyceride fraction from the columns with the unsieved Florisil (mesh 60-100) than from the columns with Florisil containing the finer particles. These observations prove that the use of the finer mesh is advantageous.

Recoveries of lipids from Florisil column

The above experiments led to the selection of small columns containing 4 g of Florisil of mesh about 100 hydrated to 8%. The recoveries of lipids were estimated under these conditions and employing the solvent systems described in Figs. 4 and 5. The recoveries of the relevant lipids eluted with the appropriate solvent fraction are summarised in Table 5. The recoveries of cholesterol esters estimated by the digitonin precipitation method varied from 98 to 100%. The recoveries of total cholesterol varied from 97 to 106%. An additional check on recovery of cholesterol esters was carried out by adding cholesteryl palmitate 1-C^{14} to the extract of serum lipids, and by measuring the radioactivity in all fractions that were passed through the column (Table 6). The fraction (defined as that) containing the cholesterol esters contained 103% of the total radioactivity. The apparently high recovery is within the limits of the counting procedure. It is important that negligible amounts of radioactivity were found in the other eluants from the Florisil column.

TABLE 5.

Recoveries of serum lipids after separation procedure on columns containing 4 g. Florisil hydrated to 8%.

Lipid fraction	Serum No.	Charge on column mg.	Amount recovered: Eluant No. 1 mg.	(%)	Eluant No. 2 mg.	(%)	Eluant No. 1+2 (%)	Eluant No. 3 mg.	(%)	Eluant No. 2+3 (%)	Eluant No. 4 mg.	(%)	Method used for determination
Cholesterol	1.	Total 6.48	4.46		2.40			-			-		Sperry and Webb (1950)
		Ester 4.28	4.40	(104)	-			-			-		
		Free 2.20	0.06		2.40	(109)		-			-		
	2.	Total 5.25	4.23		1.68			-			-		
		Ester 3.87	4.11	(106)	-			-			-		
		Free 1.68	0.12		1.68	(100)		-			-		
	3.	Total 5.40	4.20		1.40			-			-		
		Ester 3.97	4.10	(103)				-			-		
		Free 1.43	0.10		1.40	(98)							
	4.	Total 6.00	4.50		1.86		(106)						Abell et al. (1952)
	5.	Total 21.50	15.60		7.00		(105)						
	6.	Total 22.26	15.80		5.76		(97)						
		µmoles											
Glycerides	1.	9.57	-		8.93	(93)		0.72	(8)	(101)			Carlson (1963)
	2.	3.08	-		2.84	(92)		0.20	(6)	(98)			
	3.	1.92	-		1.75	(91)		0.10	(5)	(96)			
	4.	9.90	-		99.30	(94)		0.15	(2)	(96)			
	5.	3.89	-		3.83	(100)		0.10	(1)	(101)			
	6.	6.78	-		6.60	(97)		0.31	(5)	(102)			
		µEq											
Free fatty acids (FFA)	1.	1.77	-		-			-			1.88	(106)	Dole (1956) with modification of Trout et al. (1960)
	2.	1.80	-		-			-			1.76	(98)	
	3.	1.21	-		-			-			1.18	(98)	
	4.	0.87	-		-			-			0.85	(97)	
	5.	2.20	-		-			-			2.35	(107)	Mosinger (1965)
	6.	0.84	-		-			-			0.94	(112)	

* mg. of lipids in total serum lipid extract.

Eluant No. 1. 50 ml of 4% ether in hexane; Eluant No. 2. 50 ml of 15% ether in hexane and 20 ml of 20% ether in hexane; Eluant No. 3. 20 ml of ether; Eluant No. 4. 50 ml of 4% acetic acid in ether.

TABLE 6.

The recovery of cholesteryl palmitate-C^{14} from Florisil column.

(4 g. of Florisil mesh 100)

Charge on column: Lipid extract from 3 ml. of serum (phospholipid free), and cholesteryl-palmitate-1-C^{14} (0.02 μc)

Solvents applied to column	Radioactivity in the lipid extract applied to the column cpm	Radioactivity in the eluants from the column cpm	Per cent of the radioactivity recovered
	7,700		
Hexane (20 ml.)		0	0
4% ether in hexane (50 ml.)		7,933	103.60
15% ether in hexane (50 ml.) and ether (20 ml.)		186	2.41
Acetone (20 ml.)		16	0.21
4% acetic acid in ether (50 ml.)		7	0.09
Total radioactivity recovered		8,142	106.3

The recoveries of triglycerides estimated by the method of Carlson (1963) varied from 94 to 104%. In the ether fraction 1 - 8% of the total glycerides loaded were recovered (Table 5). They were identified as diglycerides by thin-layer chromatography.

Recovery studies in relation to serum FFA are unfortunately vitiated by the relative inaccuracy ($\pm$ 10%) of the available methods of FFA estimation. It is impossible, therefore, to assert with certainty that artifacts due to losses of FFA during chromatography, or indeed addition of FFA due to partial hydrolysis of triglycerides, do not occur. In order to prove that the composition of the FFA recovered from the Florisil column is a true reflection of the composition of the FFA present in the unseparated lipid mixture, the following experiment was carried out.

The FFA were removed from the phospholipid free fraction of serum lipids by shaking with aqueous NaOH (0.05N). The isolated FFA were divided into two equal parts. One was methylated and analysed by GLC, and the other was added to the neutral lipids, partly to reconstitute the serum lipids. The reconstituted mixture was then processed in the usual way, and a comparison was made between the fatty acid compositions of the chromatographed and unchromatographed FFA by GLC Table 7a. No difference was detected, showing that passage through the Florisil column did not appear to affect the fatty acid composition of the FFA fraction in any way. This also implies

TABLE 7a.

Changes in the percentage composition of serum FFA after column chromatography on Florisil

(4g. of Florisil mesh 100 hydrated to 8%)

Charge on column: A-lipid extract from 3 ml., B-lipid extract from 6 ml. of the same serum, each re-constituted by adding half of the amount of FFA, which were previously removed by shaking with 0.05 N Na OH.

Fatty acid short hand design	The percentage composition of serum FFA			
	Before column chromatography (FFA separated by shaking with Na OH)		After column chromatography (FFA eluted from columns loaded with the re-constituted lipid extract)	
	A	B	A	B
16:0	30.1	29.6	32.0	33.0
16:1	4.2	4.0	4.3	5.1
18:0	12.5	11.9	13.0	11.6
18:1	39.1	39.7	36.9	38.3
18:2	14.1	14.8	13.8	12.0

that no breakdown of ester linkages occurs during the chromatographic separation.

An additional experiment was carried out with a mixture of standard fatty acids. The fatty acid composition of this mixture was estimated by GLC before and after the mixtures were run through two columns, one of silicic acid and one of Florisil. Solvents identical to those described for the serum lipids were applied. The results presented in Table 7b show that passage through the two columns, under the conditions described for the two duplicated experiments with the same standard mixtures, did not change the composition of the standard mixture of fatty acids in any way.

Investigations on possible selective effects on percentage composition of free and esterified fatty acids during passage through Florisil columns

When the final procedure for the fatty acid analyses was adopted, it was tested with standard mixtures of lipids to investigate whether the passage through Florisil columns changed the composition of fatty acids in the relevant lipids. Therefore, the standard mixtures of different cholesterol esters and different FFA were run in parallel through two columns, one containing Florisil mesh 100, and the other containing Florisil mesh 60-100, in both cases hydrated to 8% (solvents as in Figs. 5 and 6). The results presented in Table 8a show that the run through the Florisil

TABLE 7b.

Changes in the percentage composition of standard mixture of free fatty acids after chromatography on two columns, one with silicic acid and the other with Florisil

Silicic acid column: 2.5g. of silicic acid mesh 60-100 hydrated to 8%.
Florisil column: 4 g. of Florisil mesh 100 hydrated to 8%.
Charge on column: A - 0.4 mg., B - 1.5 mg.

Fatty acid short hand design.	The percentage composition of free fatty acids in the standard mixture			
	Before chromatography		After chromatography	
	A	B	A	B
16:0	20.2	19.6	20.5	19.1
18:0	16.8	18.3	17.8	18.8
18:1	24.7	24.5	22.5	23.9
18:2	38.3	37.6	39.2	38.3

TABLE 8a.

Changes in the percentage composition of cholesteryl fatty acids resulting from passage through the Florisil column

A - 4 g. Florisil hydrated to 8%, mesh 100.
B - 4 g. Florisil hydrated to 8%, mesh 60-100.
Charge on column: standard mixtures of cholesterol esters.

Lipid	Charge on column mg.	Fatty acid short hand design.	The percentage composition of fatty acids		
			Before chromatography	After chromatography	
				A	B
Mixture 1.	6	18:0	81.4	83.5	82.8
		20:4	18.6	16.5	17.2
Mixture 2.	9	18:0	48.8	51.4	51.0
		18:2	37.3	35.1	36.0
		20:4	13.9	13.5	13.0
Mixture 3.	2.5	18:0	62.5	64.0	62.2
		18:2	24.2	24.2	24.4
		20:4	13.3	12.8	13.4

column of either mesh did not influence the fatty acid composition of the relevant fractions.

Similar observations were made when lipids from two different sera were analysed for their fatty acid composition by application to columns with the two different mesh sizes of Florisil. The results obtained were nearly identical (Table 8b).

These results further suggest that no molecular "damage" occurs during chromatography on Florisil.

The importance of the acetone wash on the Florisil columns for the accuracy of GLC analyses of serum FFA

The application of an acetone wash to the Florisil columns following elution of cholesterol esters, triglycerides, free cholesterol and diglycerides, and before the elution of free fatty acids has been found to be absolutely necessary, since a clear spectrum of free fatty acids by the GLC analyses could not otherwise be obtained. Omission of the acetone wash leads to the appearance on the gas liquid chromatograms of a large amount of interfering components, and, in addition, the amount of the methyl esters of the relevant fatty acids was greatly reduced. These interfering components were successfully removed by the washing down of the column with 20 ml. of acetone. The dried residue of the acetone wash, after transmethylation, was analysed by GLC and found to contain all the components which interfered with the GLC analyses of the free fatty acids (Fig. 6).

TABLE 8b.

Changes in the percentage composition of serum lipid fatty acids resulting from passage through the Florisil columns.

A-columns packed with 4 g. Florisil mesh 100, hydrated to 8%.
B-columns packed with 4 g. Florisil mesh 60-100, hydrated to 8%.
Charge on column: lipid extract from 3 ml. of normal serum (serum No. 1) and from 3 ml. of hyperlipidaemic serum (serum No. 2).

	Serum No1.		Serum No 2	
Cholesteryl fatty acids	A	B	A	B
16:0	9.4	9.7	9.4	10.4
16:1	3.4	3.1	3.0	3.0
18:0	0.5	0.3	0.8	0.4
18:1	18.0	18.0	18.9	18.0
18:2	64.0	65.0	61.9	62.6
20:4	5.0	4.2	6.4	5.4
Triglyceride fatty acids				
16:0	25.7	25.0	27.6	26.6
16:0	6.7	5.5	4.8	5.1
18:0	5.1	4.9	3.9	3.8
18:1	43.2	42.6	44.1	43.6
18:2	19.3	22.0	19.6	20.1
FFA				
16:0	29.7	30.4	30.0	32.0
16:1	5.5	5.9	8.4	7.5
18:0	17.8	18.0	10.4	9.3
18:1	36.0	35.6	36.3	37.0
18:2	10.1	10.0	11.2	11.3
20:4	T	T	3.8	3.0

Fig. 6 The effect of application of acetone wash to Florisil column on the gas-liquid chromatographic tracing of methyl esters of FFA

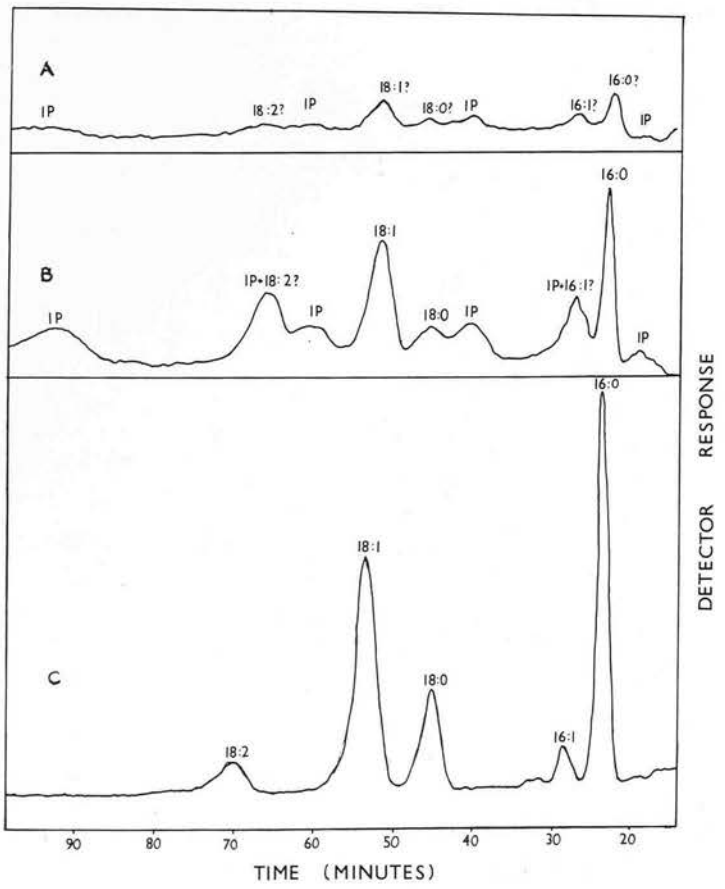
A - Gas-liquid chromatogram of methylated contamination of acetone wash

B - Gas-liquid chromatogram of methyl esters of FFA;FFA separated on Florisil column without application of acetone wash.

C - Gas-liquid chromatogram of methyl esters of FFA;FFA separated on Florisil column after application of acetone wash.

IP - Interfering peak

Operating conditions: Column characteristics and the supporting phase described in text: liquid phase: Ethylene Glycol Succinate (20%); column temperature 159°C; Argon flow rate: 45 cc/min. (A and B), 42 cc/min. (C).


A
IP
18:2?
IP
18:1?
18:0?
IP
16:1?
16:0?
IP
B
IP
IP+18:2?
IP
18:1
18:0
IP
IP+16:1?
16:0
IP
C
18:2
18:1
18:0
16:1
16:0
90
80
70
60
50
40
30
20
TIME (MINUTES)
DETECTOR RESPONSE

Neither the presence of cholesterol nor glycerides nor free fatty acids could be detected in the dry residue of the acetone wash by the appropriate colorimetric reactions. The infra-red analyses showed that (independent of the mesh used) the acetone washings contained mostly absorption bands characteristic of carboxylic groups present in cholesterol esters (frequency 1730 cm^{-1}) rather than absorption bands characteristic of triglycerides (frequency 1742 cm^{-1}; Fig. 7).

PREPARATION OF FATTY ACID METHYL ESTERS

In a few early analyses only, the methyl esters of fatty acids were prepared according to the method described by Bottcher et al., (1959), which comprised a two-step technique of saponification of lipids and of subsequent methylation of the separated fatty acids. In the majority of studies methylation was carried out according to the method of Stoffel et al., (1959) with the following modifications. Hydroquinone (about 2% of the weight of lipids) was added to the methylation medium as antioxidant. The microsublimation step was omitted,as proposed by Geyer and Bennet (1962), in order to shorten the procedure. It appeared not to be essential, reproducibility of results being satisfactory (see the section, "Reproducibility of overall procedure"), with no peaks appearing on GLC apart from those of the fatty acids.

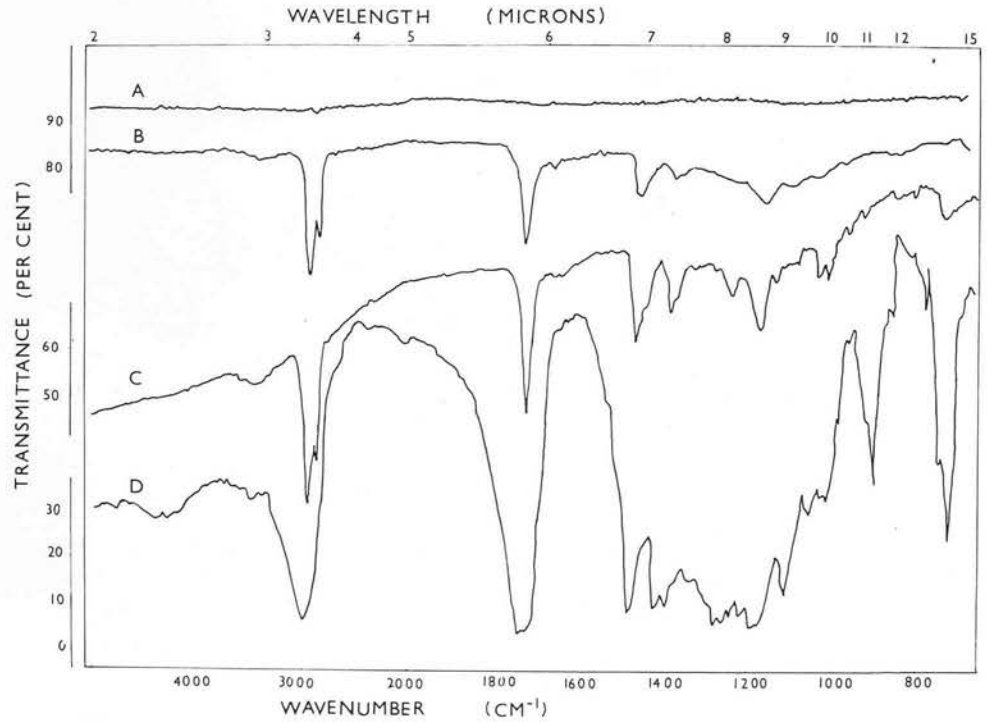
Fig. 7 The infra-red analysis of contamination of acetone wash from Florisil column

A - Solvent blank

B - Dry residue from acetone wash

C - Δ^5-cholesten-3-ol-linoleate

D - Glyceride tripalmitate


WAVELENGTH (MICRONS)
2
3
4
5
6
7
8
9
10
11
12
15
A
B
C
D
90
80
60
50
30
20
10
0
TRANSMITTANCE (PER CENT)
4000
3000
2000
1800
1600
1400
1200
1000
800
WAVENUMBER (CM-1)

The change in the methylation technique from a two-step to a one-step procedure has considerably shortened the time required, and it did not significantly alter the results of GLC analyses (Table 9).

GAS-LIQUID CHROMATOGRAPHY (GLC)

GLC analyses were performed on Pye Argon Chromatographs using ionisation detectors containing Sr^{90}. The analysing units consisted of glass columns (4 foot x 1/6 inch) packed with Ethylene Glycol Succinate polyester supported on Celite 545 (not pre-washed) of mesh 100-120. Columns packed with other liquid phases such as Reoplex 400 and Apiezon L were often used in addition. The columns with Ethylene Glycol Succinate polyester (20%) were operated at 159-160°C, columns with Reoplex 400 (10-15%) at 165-175°C and columns with Apiezon L (10%) at 180-190°C. The flow rate varied from 35 to 45 cc/minute. The detectors were operated at 1250 V.

The sample to be analysed was introduced to the column by the magnetic injection system (Pye Cat. No. 12580) in a modified way. The original glass micropipette fixed to the end of the metal rod was removed. Solution of methyl esters of fatty acids in hexane (0.2 - 0.4 ml) was pipetted into the space previously occupied by the capillary pipette, the solvent being allowed to evaporate under nitrogen. The dry esters were then introduced by the magnetic device into the column without interrupting the flow of argon.

TABLE 9.

Comparative analyses of serum lipid fatty acids, applying two different techniques for the preparation of fatty acid methyl esters

A - method of Bottcher et al. (1959), B - method of Stoffel et al. (1959).

Fatty acid short hand design.	The fraction of serum lipids	Serum No. 1		Serum No. 2		Serum No. 3	
		A	B	A	B	A	B
	PHOSPHOLIPID FATTY ACIDS						
16:0		31	28	31	32	27	26
16:1		2	2	1	2	1	1
18:0		16	17	17	17	18	16
18:1		16	15	19	20	16	17
18:2		22	24	21	21	26	28
20:4		12	13	9	8	11	12
	CHOLESTERYL FATTY ACIDS						
16:0		12	11	15	13	13	12
16:1		8	7	6	6	6	5
18:0		1	1	1	T	1	T
18:1		25	24	26	27	26	27
18:2		48	51	46	48	49	50
20:4		6	7	5	6	5	6
	TRIGLYCERIDE FATTY ACIDS						
16:0		33	32	29	29	27	26
16:1		5	4	7	7	5	4
18:0		5	6	5	4	3	4
18:1		45	44	46	45	50	50
18:2		12	13	13	15	14	15

The composition of fatty acids is expressed as a percentage of total fatty acids in the fraction.

Abbreviation: T = trace.

The quantitative GLC analyses were controlled according to recommendations of the National Heart Institute (Horning et al., 1964). Multiplication of peak height by width of half height was used for area calculations, measurements of peak width having been made with the help of a magnifying glass with fixed scale (Magniray Major, Verebles & Co. Ltd.). If changes in fatty acid composition were small, but uniformly present in all gas-liquid chromatograms, the gas-liquid chromatograms were also calculated, using triangulation and planimetry, to confirm the suspected trend. If possible, the total procedure was repeated using the initial extract.

Fatty acid standard mixtures from the National Heart Institute were used to estimate the accuracy of GLC analyses. (Mixture "C" contains myristic, palmitic, stearic, and oleic acids and is used to establish the linearity of detector responses with respect to molecular weight. Mixture "D" contains myristic, palmitic, palmitoleic, stearic and oleic acids and is used to study the column efficiency.) Quantitative results using National Heart Institute fatty acid standard mixtures ("C" and "D") agreed with the stated composition with a relative error of less than 3% for major components (more than 10% of total mixture), and less than 10% for minor components (less than 10% of total mixture). A standard mixture was always run as an external standard between the analyses of serum fatty acid methyl esters.

Each final extract of fatty acid methyl esters was analysed

by GLC on at least three occasions, often using two different liquid phases. During the study, when sequential changes were investigated, care was taken that calculations were made from gas chromatograms from the same analysing unit, operated under standard conditions. Care was taken also that the amount of sample loaded was as similar as possible on each occasion, since it is known that the response of detectors to increases in fatty acids is often not linear but differs predictably for components with different retention times. Particularly erratic results could be obtained for the palmitic and arachidonic acids when these precautions were not strictly adherred to.

Since the amount of eicosanoic and docosanoic acids in the phospholipid fraction, and of linolenic acid in the cholesterol ester fraction, as well as of pentadocosanoic in both phospholipid and cholesterol ester fractions were small, and since no significant changes were observed in any of the clinical investigations, they have not been included in the data presented. Myristic acid is usually present in phospholipids in trace amounts, in cholesterol esters to the extent of only 0.5 - 2% and in triglycerides and free fatty acids to the extent of only 2 - 4%. It was not uniformly altered during the investigations; therefore, it was also omitted from the calculations because of the relatively high standard error involved. Linolenic acid was calculated from chromatography on columns packed with Reoplex 400 and not from those packed with ethylene glycol succinate, where it

coincides with eicosanoic acid.

SOURCE OF REFERENCE COMPOUNDS

Most of the compounds used were supplied by Sigma Chemical Company Ltd., London. Compounds from other sources were as follows:-

Methyl arachidonate	Roche Products Ltd., Welwyn Garden City, England
Cholesterol-palmitate-1-C^{14}	The Radiochemical Centre, Amersham
Florisil	Floridin Company, Pittsburgh, U.S.A.
Silicic acid	Koch-Light Laboratories Ltd. Colnbrook, Bucks. England
Reoplex 400) Ethylene Glycol Succinate)	W.G. Pye & Co. Ltd., Cambridge, England.

REPRODUCIBILITY OF THE OVERALL PROCEDURE ADOPTED

The reproducibility of the overall procedure, starting from the initial total lipid extract, was estimated by ten complete analyses of one serum. Table 10 demonstrates the reproducibility of the overall procedure. For those components whose percentage composition lies between 25 and 50% of the total, the coefficient of variability* is 4-4.5.

The cholesteryl fraction is an exception. Cholesteryl oleate and palmitate had relatively higher coefficients of variability: 6.14 and 5.61 respectively. Cholesteryl palmitate is present in the same concentrations as phospholipid arachidonate, although

* The coefficient is expressed as percentage of the mean.

TABLE 10.

Reproducibility of the analytical procedure.

Replicate analysis of one serum.

Lipid fraction	Fatty acid short hand design.	Mean percentage composition	Range	Number of observations	Standard deviation	Coefficient of variability %
Phospho-	16:0	31.1	29.0-33.0	10	1.15	3.70
lipid	16:1	1.5	1.1-2.0	10	0.33	21.90
Fatty	18:0	16.1	15.0-17.0	10	0.66	4.12
Acids	18:1	14.0	13.4-15.1	10	0.63	4.49
	18:2	25.7	24.4-28.0	10	1.10	4.22
	20:3	1.6	1.3-2.0	10	0.33	20.30
	20:4	10.1	9.6-11.0	10	0.42	4.15
Choleste-	16:0	9.8	9.2-10.7	8	0.55	5.61
ryl	16:1	3.3	2.9-3.5	8	0.21	6.27
Fatty	18:0	0.7	0.5-0.9	8	0.15	21.30
Acids	18:1	18.7	17.4-20.5	8	1.15	6.14
	18:2	59.5	58.2-61.4	8	0.96	1.61
	20:4	6.5	5.8-7.3	8	0.49	7.55
Tri-	16:0	26.1	25.0-27.2	8	0.78	2.79
glyceride	16:1	5.6	5.1-6.4	8	0.46	8.27
Fatty	18:0	3.3	2.8-3.8	8	0.36	10.90
Acids	18:1	46.6	45.8-47.1	8	0.96	2.00
	18:2	18.2	17.1-19.8	8	0.83	4.50
	20:4	T	- -	8	T	-
Free	16:0	30.6	29.0-32.4	10	1.09	3.56
Fatty	16:1	4.7	4.0-5.4	10	0.46	9.15
Acids	18:0	13.5	12.9-14.1	10	0.58	4.36
(FFA)	18:1	39.4	38.2-41.3	10	0.85	2.15
	18:2	11.5	10.9-12.1	10	0.35	3.04

the coefficient of variability for the latter is lower (C = 4.15). Components between 3 and 10% of the total fatty acids have coefficients of variability proportionally greater (C= 6.14 and C= 10.90) in all lipid fractions studied.

DISCUSSION OF THE METHODOLOGICAL STUDY AND OF THE OVERALL TECHNIQUE ADOPTED

The overall procedure described for analyses of serum fatty acids in four lipid fractions requires approximately two days, and, in this time, simultaneous analyses of two serum samples can be carried out by one person. The separation of lipids into four chemical classes can be performed in 2-2$\frac{1}{2}$ hours by applying the two-column system here described.

Since column adsorption chromatography was first used for lipid analyses by Trappe (1940), who separated the hepatic cholesterol esters from triglycerides on alumina columns, this technique has been widely employed. The most widely used adsorbent for this purpose is silicic acid, since Borgström (1952) demonstrated that it exhibits a greater resolving power than does alumina. Hirsh and Ahrens (1958) elaborated a standard technique for the separation of lipids on silicic acid columns, and Ikels (1961) elaborated a modification of this method on a micro-scale.

The use of a single silicic acid column required laborious

preparation of the silica gel, together with complicated chromatographic equipment involving temperature and pressure control. Even in the micromodification (Ikels, 1961), the separation of four lipid classes required 5 hours, with additional time necessary for the preparation of the silicic acid. The use of a single silicic acid column for the separation of various lipid classes has been criticised (Böttcher et al., 1959) on the grounds that FFA could not be separated from triglycerides and sterols, and that phospholipids could not be recovered quantitatively. Irreversible adsorption, premature elution and artifact formation on silicic acid columns were earlier reported (see the paragraph in this section entitled "Consideration of analytical techniques). The separation of phospholipids and of FFA was recommended before chromatography on silicic acid (Böttcher et al., 1959).

The use of Florisil as an adsorbent for the separation of lipids by Carrol (1960) provided the prospect of a more rapid separation technique, although the separation of phospholipids before the chromatography on Florisil was also shown to be essential (Carrol 1960).

The investigations reported here set out to study optimal conditions for the separation of serum lipids on Florisil and for the subsequent analysis of their fatty acid components by GLC. Under the conditions described, no untoward effects upon the

fatty acid molecules were observed. It has been demonstrated that Florisil columns containing 4 g of Florisil hydrated to 8% can successfully separate lipids from 3 ml of serum within 60 - 70 minutes for patients with normal or only ~~markedly~~ moderately elevated serum lipids. For patients with extreme hyperlipidaemia, however, it is advisable to use an extract prepared from less than 3 ml of serum. It has been shown also that the FFA can be successfully separated from other lipids on Florisil columns, provided that the columns are cleared before the elution of this fraction by an acetone wash to remove impurities (probably products of cholesterol ester oxidation). The removal of water from the column may also play some role, the nature of which is unknown.

It appears that the techniques described here have significant advantages when compared with other methods used for the separation of FFA from other lipids. The use of an ion exchange resin (Amberlite IRA 400), as proposed by Savary and Desnuelle (1954) and Borgström (1952), implies the use of another column chromatographic separation, and was reported to lead to partial decomposition of fatty acids (Bottcher et al., 1959). An alternative method of shaking lipid extracts with aqueous KOH and recovery of the FFA after acidification of soaps, was preferred by Bottcher and his co-workers, but there are difficulties in the separation of the aqueous and organic phases due to the formation of emulsions.

Since the Florisil column is not capable of separating phospholipids and individual neutral lipids (Carrol, 1960) simultaneously, the phospholipids must be removed prior to chromatography on Florisil.

In the present study a modification of the method of Borgstrom (1952) is presented. It has been demonstrated that the hydration of silicic acid by the technique described has increased the capacity of the silica gel to adsorb serum phospholipids by a factor of two. It was accepted that adsorption usually attains its maximum when all free water is removed (Trueblood and Malmberg, 1954). However, it has already been demonstrated (Kay and Trueblood, 1949) that in some conditions an unusual increase of adsorptive strength of silica gel on columns occurs with an increased amount of adsorbed water. These authors explained this behaviour either on the basis of the hydrogen bonding capacity of water or by the overlap of the phenomena of adsorption and partition chromatography on hydrated gel. It is likely that a similar explanation may apply to the chromatographic properties of silicic acid toward the polar molecules of the phospholipids observed here.

As compared to the methods of Van Beers et al., (1958), which employ the dialysis of lipids for 24 hours across a rubber membrane, the method described here appears to be better suited

for routine analysis by virtue of being less time-consuming. The method of Van Beers et al., (1958) has been criticised, in addition, because loss of some cholesterol ester retained on the rubber membrane has been observed (Böttcher et al., 1959).

It has been suggested (Böttcher et al., 1959) that active silicic acid deteriorates, but this was not observed in the present study on serum lipids, probably because of the use of small columns, or because of the use of coarser mesh. The differences observed between the fatty acid composition of the lipid fractions by the application of either dried or hydrated silicic acid were negligible. It is, however, interesting that these differences were considerable when mixtures of free or cholesteryl fatty acids were passed through the columns. In this case, the dried, but not the hydrated, adsorbent led to alterations in the fatty acid composition of the relevant mixtures, indicating a greater risk of untoward effects on columns with the dried adsorbent. It is likely that the absence of these effects during chromatography of serum lipids may be attributed to the fact that the phospholipids adsorbed on silicic acid columns acted in this case as a 'de-activator'.

The procedure adopted here gave satisfactory recoveries and reproducibility. Although the time taken for a single analysis has been shortened as compared to that required by other methods

described, it does not mean that the entire method, including GLC analysis, is simple. It remains laborious, and will give good results only in the hands of an experienced and careful worker.

RESULTS

PRESENTATION OF DATA

As an introduction to the study, the composition of fatty acids in healthy men is compared with similar data from five other laboratories (Tables 11 and 12). The day-to-day variation in the percentage composition is calculated by an approximate method from paired estimations in subjects kept on a free diet (Table 13).

The actual study, which includes investigations on effects produced by different influences upon fatty acid mobilisation, is described separately for each lipid fraction (Tables 14 - 21). The effects of hormonal influences and of fasting are presented in separate tables (Tables 14, 16, 18 and 20) as are the effects of the diseases studied (Tables 15, 17, 19 and 21). The differences between the data obtained in diseased and healthy groups were processed statistically by Student's *t* test. The changes which occurred after treatment with hormones, and those due to fasting, were processed by the application of Student's *t* test to paired comparisons.

The detailed results obtained from these studies are included in the Appendix, Tables 1 - 10.

THE PERCENTAGE COMPOSITION OF SERUM LIPID FATTY ACIDS IN HEALTH

When the data concerning the composition of serum lipid fatty acids in healthy males were compared with those for

individuals of similar age reported from five other laboratories some differences were noted (Tables 11 and 12).

The results of this study provide the highest values for the mean percentage of free stearic acid; the levels for free oleic acid are also higher than those observed by some other authors, but are similar to those reported by Caren and Corbo (1966).

The present results are similar to those of the Swedish group, Hallgreen et al., (1960), in respect of serum triglyceride fatty acids, and similar to those of the Californian group, Caren and Corbo (1966), in respect of serum cholesteryl fatty acids. The only difference in the phospholipid fatty acids in that the present observations show slightly lower values for phospholipid palmitate than those observed by other authors.

THE DAY-TO-DAY VARIATION IN THE PERCENTAGE COMPOSITION OF SERUM LIPID FATTY ACIDS

These values were calculated from random subjects who had taken a free diet, and were investigated after an overnight fast on two different occasions at intervals of between 1 day and 3 months.

The results (Table 13) show that the fraction which shows the largest day-to-day variation is the serum FFA. The percentage of FFA oleic acid is the most variable, and that of FFA linoleic acid is least variable.

The stability of the pattern of serum triglycerides, cholesterol esters and phospholipids is noteworthy.

TABLE 11.

The percentage fatty acid composition of serum FFA in normal healthy subjects.

(Data from five investigations)

Reference	Lindgren et al. (1961)	Dole et al. (1959)	Schrade et al. (1960)	Caren and Corbo (1966)	The present study
No. of subjects investigated	4	18	15	5	10
Sex and age range	"young males"	not specified	males aged 18-42	3 men and 2 women (middle-aged)	males aged 26-51
Fatty acid short hand design.					
16:0	25	23 ± 2.8	27 ± 0.8	26 ± 0.9	27 ± 6.9
16:1	4	2 ± 0.9	7 ± 0.6	5 ± 0.4	4 ± 1.8
18:0	10	13 ± 2.6	15 ± 0.7	8 ± 0.6	20 ± 5.1
18:1	26	33 ± 6.3	25 ± 0.8	38 ± 0.8	37 ± 5.9
18:2	16	15 ± 4.1	13 ± 0.9	18 ± 1.0	11 ± 3.0
Region	California	New York	Germany	California	Scotland

All results were recalculated as percentages for the components listed, and are expressed as Mean ± 1 S.D.

TABLE 12.

The percentage fatty acid composition of serum esterified lipids in normal healthy subjects.

(Data from seven investigations)

Reference	Hallgreen et al.(1960)	Lingren et al.(1961)	Schrade et al.(1961)	Lawrie et al.(1960)	Caren and Corbo (1966)	Laudat et al.(1966)	The Present Study
No. of subjects investigated	5	7	15	13	20	5	12
Sex and age range	males aged 23-38	males "young"	males aged 18-42	males	mixed sex aged 17-69	mixed sex aged 25-55	males aged 26-51
Fatty acid short hand design.			TRIGLYCERIDE FATTY ACIDS				
16:0	29	32	32 ± 1.0	30 ± 6.2	27 ± 0.9	27 ± 3.6	28 ± 4.3
16:1	7	4	9 ± 0.4	10 ± 2.5	4 ± 0.2	5 ± 2.2	6 ± 1.1
18:0	5	5	4 ± 0.4	5 ± 2.2	5 ± 0.3	3 ± 1.1	5 ± 0.3
18:1	47	42	41 ± 0.8	37 ± 11.3	43 ± 0.7	44 ± 1.8	47 ± 4.4
18:2	13	17	14 ± 0.7	17 ± 6.0	18 ± 0.9	12 ± 3.9	14 ± 2.8
			PHOSPHOLIPID FATTY ACIDS				
16:0	33	35	35 ± 1.2	34 ± 7.3	32 ± 1.1	33 ± 2.4	29 ± 4.3
18:0	14	15	14 ± 0.6	13 ± 3.4	15 ± 0.6	16 ± 3.1	17 ± 1.5
18:1	17	13	17 ± 0.8	21 ± 3.6	13 ± 0.5	16 ± 3.8	17 ± 2.3
18:2	24	23	24 ± 0.8	22 ± 5.5	24 ± 0.7	18 ± 5.0	25 ± 3.3
20:4	10	10	9 ± 0.5	8 ± 3.0	10 ± 0.7	11 ± 5.3	11 ± 1.6
			CHOLESTERYL FATTY ACIDS				
16:0	12	16 ± 4.0	13 ± 1.1	12 ± 0.3	12 ± 1.2	12 ± 2.5	10 ± 1.2
16:1	5	7 ± 2.0	7 ± 0.4	3 ± 0.2	3 ± 1.0	5 ± 1.2	4 ± 1.1
18:0	1	4 ± 1.9	3 ± 0.7	2 ± 0.1	1 ± 0.2	1 ± 0.5	1 ± 0.2
18:1	25	26 ± 6.3	20 ± 0.6	20 ± 0.5	24 ± 1.6	21 ± 4.7	20 ± 0.5
18:2	50	42 ± 13.0	52 ± 1.1	55 ± 0.8	48 ± 5.3	53 ± 8.2	56 ± 5.7
20:4	6	5 ± 3.4	5 ± 0.6	7 ± 0.3	7 ± 1.7	5 ± 4.2	7 ± 1.4
Region	Sweden	California	Germany	Scotland	California	France	Scotland

All results were recalculated as percentages for the components listed, and are expressed as Mean±1 S.D.

TABLE 13.

Day-to-day variations in the percentage composition of fasting serum fatty acids.

(The mean differences were calculated from the estimations carried out on two occasions in individuals taking a free diet)

Group of lipid	Free fatty acids (FFA)			Triglyceride fatty acids			Phospholipid fatty acids			Cholesteryl ester fatty acids		
No. of subjects	11			20			18			18		
Fatty acid short hand design.	Mean value %	Mean difference	S.D. mean difference	Mean value %	Mean difference	S.D. mean difference	Mean value %	Mean difference	S.D. mean difference	Mean value %	Mean difference	S.D. mean difference
16:0	27	3.2 ±	2.6	28	1.4 ±	1.2	32	2.2 ±	1.9	11	0.6 ±	0.8
18:0	14	2.0 ±	1.9	4	0.6 ±	0.7	16	1.2 ±	1.2	1	-	-
18:1	42	3.9 ±	3.8	49	1.7 ±	1.1	17	1.1 ±	0.9	22	1.2 ±	1.5
18:2	10	1.0 ±	1.0	13	1.6 ±	1.9	21	2.1 ±	1.4	52	1.4 ±	1.0
20:4	-	-		-	-		11	0.9 ±	0.9	7	1.4 ±	1.1

CHANGES IN THE COMPOSITION OF SERUM FREE FATTY ACIDS (FFA) IN RESPONSE TO DIFFERENT FACTORS INFLUENCING FATTY ACID MOBILISATION

Thyroid hormone

(i) Thyroid hormone treatment in normal and hypothyroid subjects

The serum FFA concentrations were not uniformly changed in the two normal men under study. The serum FFA increased in one, and decreased in the other man during the 7-day period of administration of 100 μg of LT_3 (Appendix Table 1). Since this experiment was carried out early in the study, it does not include information about the composition of serum FFA.

Significant changes were observed in the hypothyroid patients following treatment with thyroid hormone (Table 14). The fasting levels of serum FFA increased as a result of the treatment ($P < 0.05$). In the treated state, as compared to the untreated, higher percentages of FFA oleic acid ($P < 0.005$) and lower percentages of saturated acids (mainly palmitic acid, $P < 0.005$) were found (Table 14, Appendix Fig. 1, Appendix Table 2). Thus, after thyroid hormone therapy the FFA showed an increase in monounsaturated and a decrease in saturated fatty acids (Fig. 8).

(ii) Antithyroid therapy in thyrotoxic subjects

Changes in the concentration and composition of serum FFA were for the most part opposite to those observed when thyroid therapy was given to hypothyroid subjects (Appendix Table 3).

TABLE 14.

Changes in percentage composition of serum free fatty acids (FFA) in response to factors influencing fatty acid mobilisation

Factors influencing fatty acid mobilisation	No. of subjects	No. of observations	Serum FFA concentration μEq/l			Serum FFA composition (%)											
						16:0			18:0			18:1			18:2		
			Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference
Thyroid hormone therapy in hypothyroid subjects (average fasting values in treated compared with untreated state)	9	16	751 (±337)	947 (±326)	+196[a] (±185) P<0.05	32 (±2.3)	28 (±0.9)	-3.8 (±2.1) P<0.005	15 (±3.1)	14 (±1.9)	-0.4 (±2.9)	40 (±3.9)	46 (±3.9)	+5.8 (±3.7) P<0.005	8 (±0.7)	7 (±1.9)	-1.3 (±1.1) P<0.01
Antithyroid therapy in thyrotoxic subjects (average fasting values in treated compared with untreated state)	4	8	887 (±115)	676 (± 75)	-211 (±112) P<0.05	26 (±1.6)	30 (±1.0)	+4.3 (±2.5) P<0.05	12 (±2.2)	15 (±3.7)	+3.0 (±2.3)	49 (±1.3)	42 (±3.9)	-6.9 (±3.8) P<0.05	8 (±2.8)	8 (±1.5)	0 (±1.2)
HGH injection (changes observed 8 hours after HGH injection during a period of fasting)	3	3	743 (+258)	1463 (±246)	+720 (± 51) P<0.005	29 (±0.7)	21 (±5.0)	-8.0 (±9.2)	21 (±2.2)	14 (±1.6)	-7.7 (±5.7)	39 (±8.5)	55 (±3.0)	+16.3 (±6.1) P<0.05	8 (±3.6)	8 (±2.4)	0 (±2.5)
Noradrenaline infusion (changes observed after a 15-minute noradrenaline infusion)	6	6	535 (±132)	846 (±100)	+311 (±222) P<0.02	28 (±2.3)	26 (±3.6)	-2.3 (±2.1)	17 (±2.1)	13 (±2.5)	-3.0 (±1.1) P<0.005	44 (±2.2)	48 (±3.3)	+3.8 (±2.3) P<0.01	6 (±1.2)	8 (±1.3)	+1.7 (±1.4) P<0.05
Fasting (changes observed after prolongation of overnight fast by 8 hours)	12	12	688 (±239)	976 (±269)	+288 (±275) P<0.005	25 (±6.7)	27 (±5.4)	+1.7 (±2.0)	18 (±11.4)	14 (±2.6)	-4.4 (±5.5) P<0.02	43 (±5.6)	47 (±4.4)	+3.0 (±4.4) P<0.05	11 (±2.6)	9 (±2.1)	-1.3 (±1.7) P<0.025

[a] Serum FFA concentrations were estimated in 7 subjects

TABLE 15.

The percentage composition of serum free fatty acids (FFA) in normal healthy subjects, in subjects with ischaemic heart disease and in subjects with different hormonal disorders.

No.	Group of subjects	No. of subjects	Serum FFA concentration mEq/l. Mean	SD	Serum FFA composition (%) 16:0 * Mean	SD	18:0 * Mean	SD	18:1 * Mean	SD	18:2 * Mean	SD
No. 1a	Normal males aged 26-51	10	490	± 34	27	± 6.9	20	± 5.1	37	± 5.9	11	± 3.0
No. 1b	Normal females aged 50-60	4	620	± 58	26	± 3.1	15	± 3.1	44	± 2.6	10	± 3.1
No. 2**	IHD, chronic, males aged 33-51	14	618	± 202	28	± 4.4	15	± 4.1	41	± 2.8	10	± 2.5
No. 1a - No. 2							P < 0.02		P < 0.05			
No. 3	IHD acute, post myoc. infarction 24-32 hrs. 7 males, 1 female aged 45-62	8	804 (levels on the previous day 929	± 120 ± 110)	27	± 3.1	13	± 1.6	47	± 3.6	8	± 1.2
No. 1 - No. 3							P < 0.005		P < 0.001		P< 0.002	
No. 2 - No. 3									P < 0.05			
No. 4	Hyperthyroid 3 males, 2 females, aged 50-60	5	843	± 118	24	± 2.8	12	± 2.9	50	± 2.4	8	± 2.2
No. 5	Hypothyroid 1 male, 8 females, aged 50-60	9	751	± 337 [a]	32	± 2.4	15	± 2.9	39	± 4.0	8	± 2.2
No. 4 - No. 5							P < 0.001		P < 0.001			
No. 1b - No. 5									P < 0.005			
No. 6	Acromegaly 3 females aged 50-60	3	386	± 186	28	± 6.9	24	± 4.0	36	± 2.8	9	± 2.1
No. 1b - No. 6							P < 0.025		P < 0.02			

* Fatty acid short hand designation.

** Patients with angina pectoris or 2 months-2 years after myocardial infarction.

The P values in frames were calculated from groups of subjects of similar age and the same sex.

[a] Serum FFA concentrations were estimated in 7 subjects.

Fig. 8 The percentages of FFA and triglyceride saturated and monounsaturated acids in relation to thyroid function and in response to treatment with thyroid hormones or antithyroid therapy (Mean ± 1S.D.)

Saturated acids: palmitic and stearic acids
Monounsaturated acids: oleic and palmitoleic acids.


hypothyroid subjects


euthyroid subjects

" " (middleaged males)

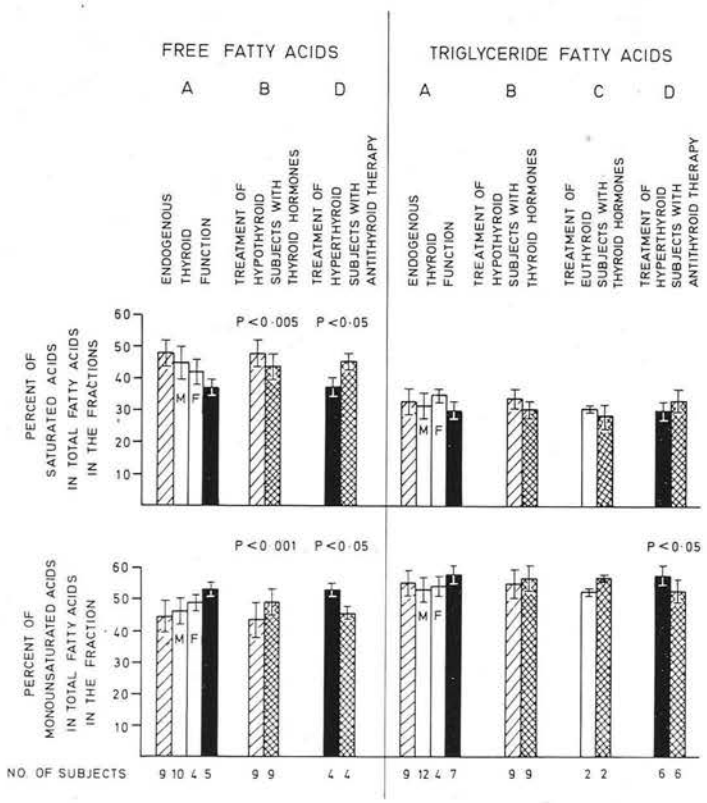
" " (postmenopausal females)


hyperthyroid subjects


after treatment

B and C changes accompanied by increases in serum FFA concentrations

D changes accompanied by decreases in serum FFA concentrations


FREE FATTY ACIDS
TRIGLYCERIDE FATTY ACIDS
A
B
D
A
B
C
D
ENDOGENOUS THYROID FUNCTION
TREATMENT OF HYPOTHYROID SUBJECTS WITH THYROID HORMONES
TREATMENT OF HYPERTHYROID SUBJECTS WITH ANTITHYROID THERAPY
TREATMENT OF EUTHYROID SUBJECTS WITH THYROID HORMONES
PERCENT OF SATURATED ACIDS IN TOTAL FATTY ACIDS IN THE FRACTIONS
PERCENT OF MONOUNSATURATED ACIDS IN TOTAL FATTY ACIDS IN THE FRACTION
P<0·005
P<0·05
P<0·001
M
F
NO. OF SUBJECTS
9 10 4 5
9 9
4 4
9 12 4 7
9 9
2 2
6 6

Antithyroid therapy significantly lowered the levels of serum FFA ($P < 0.05$). The percentage level of FFA oleic acid decreased ($P < 0.05$) and the percentage level of FFA palmitic acid increased ($P < 0.05$; (Table 14). The average decrease in the concentrations, as well as the average percentage decrease of oleic and increase of palmitic acid, were nearly of the same magnitude as the corresponding changes observed in hypothyroid patients treated with thyroid hormones. Thus, after antithyroid therapy, the FFA showed a decrease in monounsaturated acids and an increase in saturated fatty acids (Fig. 8).

Diseases of the thyroid

The difference in the average levels of serum FFA, as recorded in the groups of hyper- and hypothyroid patients, were not significant. The composition of FFA in these two groups, however, differed significantly, and independently of age and sex. The FFA of hypothyroid patients had a higher percentage of palmitic ($P < 0.001$) and lower percentage of oleic acid ($P < 0.001$) than those of hyperthyroid patients (Table 15).

Of all the groups studied, hypothyroid patients had the highest percentage of free palmitic acid. The values were significantly higher than those of normal middle-aged males and those of older females. This observation on the effects

of spontaneous hypothyroidism is in agreement with that made on the effect of antithyroid therapy. Similarly antithyroid therapy results in higher percentages of FFA palmitic acid.

Of all the groups studied, hyperthyroid patients had one of the highest percentages of free oleic acid. Similarly high levels were seen in patients after myocardial infarction, (Table 15). This observation on the effects of spontaneous hyperthyroidism is in agreement with the observation made on the effects of thyroid hormone therapy in hypothyroid subjects, where thyroid hormone therapy has also led to higher percentages of free oleic acid.

Human growth hormone (HGH)

(i) Effects produced within hours after the injection

The evidence on the effects of HGH given by intramuscular injection derives from two experiments. In one, which was performed in the early course of the study, the composition of esterified fatty acids only, but not of the FFA, was investigated. In this early experiment, each daily injection was followed by a normally distributed and carefully controlled balanced diet. The serum FFA were measured 3 hours after the administration of HGH (approximately 3 hours after the first meal) and 7 hours after the HGH injection (approximately 4 hours after the second meal). The results illustrated in Fig. 9 show that almost all daily levels are elevated during the period of HGH administration.

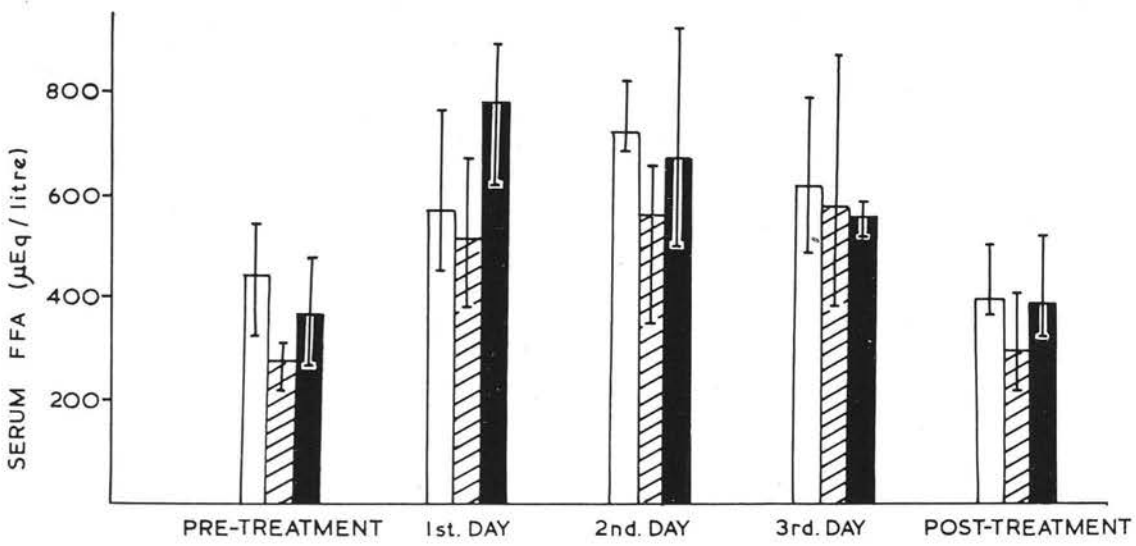
Fig. 9 Changes in the daily levels of serum FFA concentrations in response to HGH injections

(Mean values from three patients, who received three daily HGH injections followed by meals , and range of values)

Fasting blood sample (taken at 9 a.m.)

Blood sample taken 3 hours after HGH injection and 3 hours after the first meal (12 noon)

Blood samples taken 7 hours after the HGH injection and 4 hours after the second meal (4 p.m.)


SERUM FFA (µEq / litre)
800
600
400
200
PRE-TREATMENT
1st. DAY
2nd. DAY
3rd. DAY
POST-TREATMENT

The highest levels occurred 7 hours after the first two daily injections.

The second experiment gives more information on the "short-term" effects of HGH injection, since both the concentrations and composition of FFA were investigated hourly after the injection. In this experiment, the first of the two daily injections (given after an overnight fast) was followed by a further 8 hours of fasting, during which time the short-term effects of HGH injection were compared with the effects of fasting alone. The results illustrated on Fig. 10 (and in Appendix Table 6) show that all three men studied had a marked increase in serum FFA, and these increases were most marked 8 hours after injection. These levels were on average twice as high as those observed in the morning. The FFA most affected by this rise was oleic acid: 8 hours after injection, in the three men the increase of oleic acid concentration alone was responsible for 74, 82 and 61 per cent of the rise in serum total FFA respectively.

(ii) Effects of daily injections of HGH

The "long-term" effects of HGH injection were studied 24 hours after the administration of the last injection, following an overnight fast. When the values so obtained were compared with the fasting values during the pre-treatment period, various responses were observed with respect to the fatty acid concentrations and composition (Appendix Table 5).

Fig. 10 Changes in percentage composition of serum FFA in relation to changes in absolute concentrations 4 and 8 hours after HGH injection followed by prolonged fasting

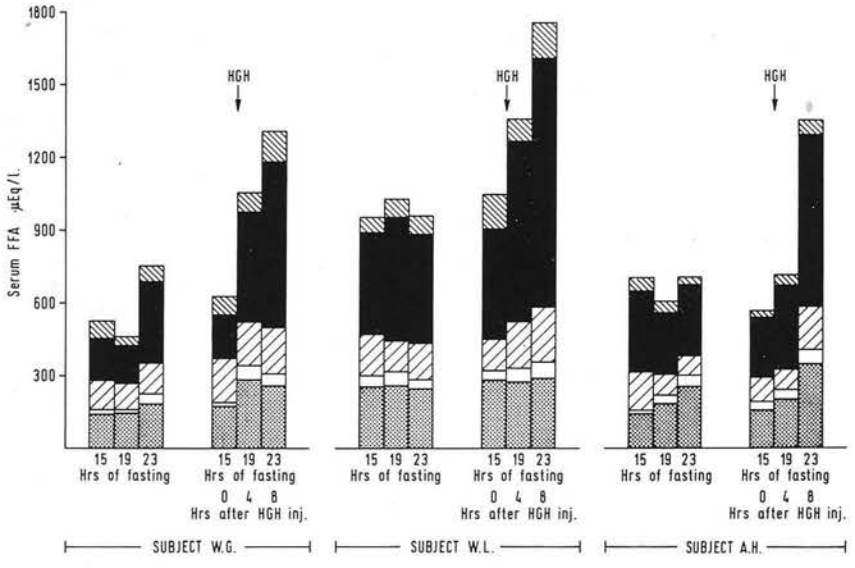
palmitic acid

palmitoleic acid

stearic acid

oleic acid

linoleic acid


1800
1500
1200
900
600
300
Serum FFA µEq/l.
HGH
HGH
HGH
15 19 23
Hrs of fasting
15 19 23
Hrs of fasting
0 4 8
Hrs after HGH inj.
SUBJECT W.G.
15 19 23
Hrs of fasting
15 19 23
Hrs of fasting
0 4 8
Hrs after HGH inj.
SUBJECT W.L.
15 19 23
Hrs of fasting
15 19 23
Hrs of fasting
0 4 8
Hrs after HGH inj.
SUBJECT A.H.

In the group of patients investigated early in the study, evidence is available only on the concentrations of serum FFA. Of the four patients in this group, elevated serum FFA levels were noted in three men during the first two days of the administration of HGH.

In three patients studied later (who received a first injection followed by an 8 hour fast), one showed an elevated serum FFA level 24 hours after the first injection, and none 24 hours after the second injection. A decrease in the percentage of free stearic acid was noted in all three men 24 hours after the first and second injections and in two men 48 hours after the second injection. An increase in the percentage of FFA oleic acid was noted in two men 24 hours after the first and 24 hours after the second injection. These changes were present in spite of the fact that the total levels of serum FFA had returned to normal, or indeed had fallen below normal levels (Appendix Table 6b).

(iii) Acromegaly

The serum FFA concentrations were unusually low in two out of the three patients with acromegaly (Appendix Table 7); therefore, the average levels in this group were lower than in any other group under study. There was a lower than normal percentage of FFA oleic acid ($P < 0.02$), and a higher than normal percentage of FFA stearic acid ($P < 0.025$, Table 15).

Noradrenaline

During the infusion of 100 µg of noradrenaline, over a period of 15 minutes the serum FFA increased ($P < 0.02$) and changed in composition significantly. The FFA oleic and linoleic acids were increased ($P < 0.01$ and $P < 0.05$ respectively) and the FFA stearic acid was decreased ($P < 0.005$, Table 14, Appendix Table 8) There was an increase in the monounsaturated and a decrease in the saturated acids (Fig. 11).

Fasting

As shown in the data for individual subjects (Appendix Table 9), the changes in fatty acid composition were not related to the original fasting concentrations, either of serum FFA or esterified lipids; therefore, all subjects were regarded as one group. The prolongation of an overnight fast by 8 hours induced a rise in serum FFA concentrations ($P < 0.005$), an increase in the percentage of FFA oleic acid ($P < 0.05$), a decrease in the percentage of FFA stearic acid ($P < 0.02$), and a decrease in the percentage of FFA linoleic acid ($P < 0.025$, Table 14, Appendix Table 9, Appendix Fig. 2). Thus the prolongation of fasting, as all the other stimuli of fatty acid mobilization described above, caused the FFA monounsaturated acids to rise and the FFA saturated acids to fall (Fig. 11).

Fig. 11 Changes in percentages of FFA saturated and monounsaturated acids in response to treatments influencing fatty acid mobilisation and in relation to ischaemic heart disease and diseases of hormonal disorder (**Mean 1± S.D.**)

Saturated acids: palmitic and stearic acids
Monounsaturated acids: oleic and palmitoleic acids.

 before treatment

 after treatment

 normal middleaged males

 normal postmenopausal females

 diseased subjects

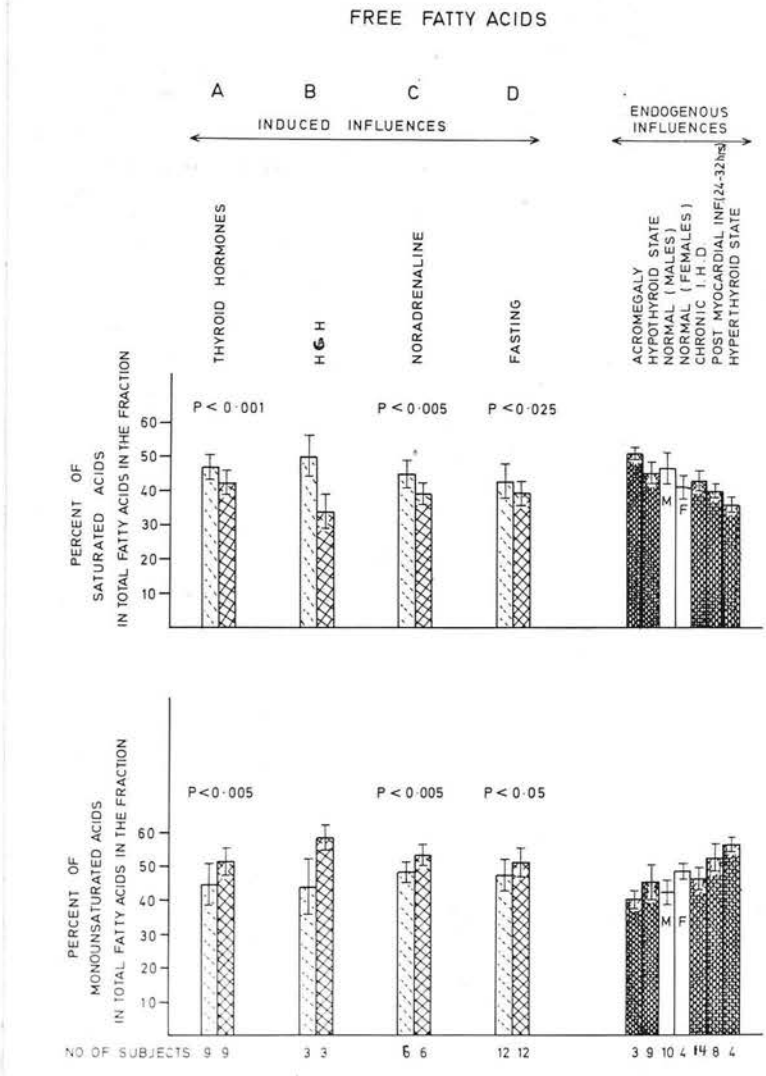
A - D Changes accompanied by increases in serum FFA concentrations:

A - After treatment of hypothyroid subjects with thyroid hormones

B - 8 hours after HGH injection followed by prolonged fasting

C - After a 15 minute infusion of noradrenaline

D - After a prolongation of overnight fast by 8 hours


FREE FATTY ACIDS
A
B
C
D
INDUCED INFLUENCES
ENDOGENOUS INFLUENCES
THYROID HORMONES
H & H
NORADRENALINE
FASTING
ACROMEGALY
HYPOTHYROID STATE
NORMAL (MALES)
NORMAL (FEMALES)
CHRONIC I.H.D
POST MYOCARDIAL INF(24-32hrs)
HYPERTHYROID STATE
PERCENT OF SATURATED ACIDS IN TOTAL FATTY ACIDS IN THE FRACTION
P < 0·001
P < 0·005
P < 0·025
PERCENT OF MONOUNSATURATED ACIDS IN TOTAL FATTY ACIDS IN THE FRACTION
P<0·005
P < 0·005
P < 0·05
NO OF SUBJECTS 9 9 3 3 6 6 12 12 3 9 10 4 14 8 4

Ischaemic heart disease (IHD)

(i) Acute myocardial infarction

Patients who had blood taken within 32 hours after an acute myocardial infarction had, on average, higher levels of serum FFA than patients with chronic ischaemic heart disease. In addition, the mean increase of FFA oleic acid (10% against 4%), and the mean decrease of FFA stearic acid (7% against 5%), were higher in patients immediately after an acute myocardial infarction than in those with IHD (Table 15). The statistical significance of these changes was at a higher level than those observed in patients with chronic IHD (Table 15). The possibility can not be excluded that the difference in results is in part due to the different ages of the two groups studied.

Comparison of patients who had recently sustained an acute myocardial infarction with those with thyrotoxicosis reveals a striking similarity in the pattern of the FFA changes.

The sequential changes between the first, second and third day after the onset of myocardial infarction were not uniform in all subjects studied. Increases in percentages of FFA oleic and decreases in percentages of palmitic acids, accompanied by lowering of serum FFA concentrations were observed in 4 out of the 8 patients under study. In two other patients (J. Co., and J. M.) there were marked changes in the opposite direction, leading to a higher degree of saturation (Appendix Fig. 3 Appendix Table 10).

These/

two patients represented clinically the most severe cases (one died on the third day, and one developed a cerebral vascular episode with hemiplegia on the second day).

(ii) Chronic ischaemic heart disease

Consistently with the higher than average levels of serum FFA in patients with IHD, percentages of serum FFA oleic acid higher than normal ($P < 0.02$) and percentages of FFA stearic acid lower than normal ($P < 0.05$) were observed in this group (Table 15).

CHANGES IN THE COMPOSITION OF SERUM TRIGLYCERIDE FATTY ACIDS IN RESPONSE TO DIFFERENT FACTORS INFLUENCING FATTY ACID MOBILISATION

Thyroid hormone

(i) Thyroid hormone treatment in normal and hypothyroid subjects

After the administration of 100 μg of LT_3 daily for seven days, to two men, one (whose FFA increased) showed a change in triglyceride composition, but neither showed any substantial change in total triglyceride level. In this one subject changes in composition were in the same direction as those described below for the hypothyroid patients following treatment with thyroid hormones: an increase in triglyceride oleate (by 5% of the total fatty acids in the triglyceride fraction) and a decrease in triglyceride stearate (by 2% of the total fatty acids in the triglyceride fraction, Appendix Table 1).

TABLE 16.

Changes in percentage composition of serum triglyceride fatty acids in response to factors influencing fatty acid mobilisation

Factors influencing fatty acid mobilisation	No. of subjects	No. of observations	Serum FFA concentration μEq/l. Before treatment	After treatment	Observed difference	Serum triglyceride concentration m mole/l. Before treatment	After treatment	Observed difference	Serum triglyceride fatty acid composition (%) 16:0* Before treatment	After treatment	Observed difference	18:0* Before treatment	After treatment	Observed difference	18:1* Before treatment	After treatment	Observed difference	18:2* Before treatment	After treatment	Observed difference
Thyroid hormone therapy in hypothyroid subjects (average fasting values in treated compared with untreated state)	9	16	751 (±337)	947 (±326)	+196[a] (±185) P<0.05	2.51 (±1.55)	1.45 (±1.57)	-1.06 (±1.08) P<0.02	28 (±3.3)	27 (±2.0)	-0.7 (±3.5)	5 (±0.9)	4 (±1.2)	-1.2 (±0.7) P<0.005	48 (±3.9)	50 (±3.5)	+1.6 (±3.9)	11 (±2.6)	10 (±2.2)	-0.7 (±3.1)
Antithyroid therapy in thyrotoxic subjects (average values in treated compared with untreated state)	6	10	952 (±195)	634 (±130)	-318 (±191) P<0.01	1.35 (±0.40)	1.90 (±0.55)	+ 0.55 (±0.41) P<0.025	26 (±2.5)	28 (±3.0)	+1.7 (±1.8)	4 (±1.6)	5 (±0.9)	+1.0 (±0.8)	52 (±2.7)	48 (±0.9)	-4.1 (±2.7) P<0.02	10 (±2.3)	12 (±3.3)	+1.6 (±2.0)
HGH injection (average fasting values observed between the 1st and 3rd day of HGH administration compared with the average fasting values of the pretreatment period)	7	17	612 (±195)	671 (±111)	+59 (±74)	1.62 (±0.5)	1.77 (±0.69)	-0.15 (±0.6)	31 (±3.1)	33 (±3.6)	+2.5 (±2.0) P<0.025	4 (±1.5)	5 (±1.0)	+0.6 (±0.5)	48 (±3.6)	48 (±3.3)	-0.2 (±1.4)	9 (±1.9)	9 (±2.2)	-0.1 (±0.0)
Noradrenaline infusion (changes observed after a 15-minute noradrenaline infusion)	6	6	535 (±132)	856 (±100)	+311 (±222) P<0.02	1.45 (±0.56)	1.63 (±0.72)	+0.02 (±0.75)	29 (±2.7)	29 (±4.2)	-0.2 (±4.4)	4 (±0.2)	4 (±0.7)	-0.3 (±0.8)	49 (±2.2)	50 (±5.3)	+1.2 (±2.7)	11 (±1.9)	12 (±0.9)	+1.0 (±2.2)
Fasting (changes observed after prolongation of overnight fast by 8 hours)	12	12	688 (±239)	976 (±269)	+288 (±275) P<0.005	1.49 (±0.56)	1.91 (±0.68)	+0.42[b] (±1.24)	29 (±3.3)	29 (±2.5)	-0.3 (±3.3)	4 (±1.2)	3 (±1.2)	-1.0 (±0.7) P<0.001	48 (±0.7)	47 (±2.8)	+0.8 (±2.4)	12 (±4.4)	11 (±3.2)	-0.5 (±2.3)

[a] Serum FFA concentrations were estimated in 7 subjects

[b] Serum triglyceride concentrations were estimated in 8 subjects

* Fatty acid short hand designation.

TABLE 17.

The percentage composition of serum triglyceride fatty acids in normal healthy subjects, in subjects with ischaemic heart disease and in subjects with different hormonal disorders.

No.	Group of subjects	No. of subjects	Serum triglyceride concentration mmole/l.		Serum triglyceride fatty acid composition (%)							
					16:0*		18:0 *		18:1 *		18:2 *	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
No. 1a	Normal males aged 26-51	12	1.14	± 0.36	28	± 3.5	5	± 3.2	47	± 4.4	14	± 2.8
No. 1b	Normal females aged 50-60	4	1.46	± 0.58	31	± 1.1	4	± 1.4	47	± 2.7	11	± 3.4
No. 2**	IHD chronic, males aged 33-51	23	1.84	± 0.90	29	± 3.6	4	± 1.1	46	± 3.3	12	± 4.2
No. 2a	" with higher serum triglyceride conc.	14	2.40	± 0.82	29	± 3.9	4	± 1.1	47	± 2.8	12	± 1.9
No. 2b	" with higher serum FFA conc. (760 ± 148 µEq/1)	10	1.47	± 1.05	30	± 2.8	4	± 1.3	49	± 2.7	11	± 2.9
No. 1a - No. 2 No. 1a - No. 2a No. 1a - No. 2b			P < 0.02 P < 0.001				P < 0.001		P < 0.05 P < 0.025			
No. 3	IHD acute, post myoc. infarction 24-32 hrs. 7 males, 1 female, aged 45-62	8	1.34	± 0.50	31	± 1.92	4	± 1.5	49	± 2.8	11	± 2.5
No. 1a - No. 3									P < 0.025			
No. 4	Hyperthyroid, 3 males, 50-60; 2 females, 50-60; 2 females, 20-30.	7	1.32	± 0.04	27	± 2.5	4	± 1.4	52	± 2.9	10	± 2.2
No. 5	Hypothyroid, 1 male, 8 females aged 50-60	9	2.51	± 1.43	28	± 3.3	5	± 1.0	49	± 4.2	11	± 2.4
No. 1a - No. 4			P < 0.05				P < 0.02		P < 0.005			
No. 6	Acromegaly 3 females aged 50-60	3	1.84	± 0.18	35	± 0.6	6	± 1.00	44	± 1.00	9	± 2.6
No. 1b - No. 6					P < 0.005							

* Fatty acid short hand designation.

** Patients with angina pectoris or 2 months-2 years after myocardial infarction.

The P values in frames were calculated from groups of patients of similar age and the same sex.

Administration of thyroid hormone to hypothyroid subjects resulted in a significant lowering of serum triglyceride concentrations ($P < 0.02$, Table 16). A statistically significant decrease occurred in the percentage of triglyceride stearate ($P < 0.005$). Although increases in triglyceride oleate were observed in 7 out of the 9 subjects (Appendix Table 2), these were not statistically significant.

(ii) Antithyroid therapy in thyrotoxic subjects

Antithyroid therapy given to thyrotoxic subjects induced significant increases in serum triglyceride concentrations ($P < 0.025$), with significant decreases in the percentages of triglyceride oleate ($P < 0.02$, Table 16).

(iii) Disease of the thyroid

Considering the wide differences in the mean values of the serum triglyceride concentrations between the hyper- and hypothyroid patients (values being twice as high in the hypo- as in the hyperthyroid patients), the differences in the fatty acid composition were comparatively small (Table 17). Of all the groups studied, the hyperthyroid group showed the highest values for mean percentage of triglyceride oleate, and these values were considerably higher than those observed in either the normal male group ($P < 0.02$) or in the normal female group ($P < 0.02$).

Human growth hormone (HGH)

(i) Effects produced within hours after the injection

No significant change in the concentrations of serum triglycerides was found within a few hours after the HGH injection in either of the two groups of patients studied. Neither were changes observed in the triglyceride fatty acid composition of fasting blood specimens taken 4 and 8 hours after the injection of HGH.

(ii) Effects of daily injections of HGH

Since the trends with respect to the esterified lipids were similar in the subjects in the two experiments all subjects were regarded as a single group with respect to the changes found 24 hours after the first, second and third injections. The amalgamation of these groups must, however, be regarded critically, since in one the first HGH injection was followed by meals and in the second by prolonged fasting. In the entire accumulated group, however, similar changes, which were noticeable in patients belonging to both groups, became statistically significant. The imperfection of this statistical approach must be stressed, and its significance must therefore be accepted as an indication of a trend only, rather than as conclusive evidence.

No significant changes were observed in triglyceride concentrations, although it appears that in the 3 older subjects, who received HGH injection on each of 3 or 4 days, the serum triglyceride concentrations were somewhat higher during the period of HGH administration (Appendix Table 5). During the

administration of HGH, the percentages of triglyceride palmitate were, on average, higher than those recorded in the pretreatment period ($P < 0.02$).

(iii) Acromegaly

Patients with acromegaly (who had elevated levels of serum HGH) showed slightly higher than normal levels of serum triglycerides. These were accompanied by a high percentage of saturated acids in the triglyceride fraction. Of the groups studied, these subjects had the highest observed values for triglyceride palmitate and stearate and the lowest values for triglyceride oleate (Table 17). The triglyceride palmitate was particularly high in acromegalic women and, in spite of the small numbers of subjects under observation, the differences were statistically significant ($P < 0.005$).

Noradrenaline

No changes were observed either in triglyceride concentration or triglyceride fatty acid composition 15 minutes after the commencement of noradrenaline infusion (Table 16).

Fasting

No significant changes were observed in serum triglyceride concentrations, but small significant decreases in triglyceride stearate ($P < 0.001$) were found when the overnight fast was prolonged by 8 hours (Table 16). Although these decreases were small, and within the range of error of the analytical

procedure, the high level of significance suggests that they are real.

Ischaemic heart disease

(i) Acute myocardial infarction

The available data on triglyceride concentrations in these patients do not show any significant differences from normal. The pattern of triglyceride fatty acids was characterised by somewhat higher percentages of oleic acid, as compared to chronic IHD (Table 17). These values increased between the first and second day or second and third day after myocardial infarction (Appendix Table 10).

(ii) Chronic ischaemic heart disease

There were no differences in triglyceride composition between the healthy group of males and males with IHD, although the diseased group had higher than average concentrations of serum triglycerides ($P < 0.02$). When however, from the entire group of diseased males, those with the higher serum FFA concentrations were selected into one group, and those with the higher serum triglyceride concentration into a second group, in these groups (each composed of a smaller number of subjects) decreases in triglyceride linoleate were found to be of statistical significance compared with normal subjects. It appears therefore that high concentrations of both serum FFA and serum triglycerides are associated with lower percentages of triglyceride linoleate (Table 17).

CHANGES IN THE COMPOSITION OF SERUM PHOSPHOLIPID FATTY ACIDS IN RESPONSE TO DIFFERENT FACTORS INFLUENCING FATTY ACID MOBILISATION

Thyroid hormone

(i) Thyroid hormone treatment in normal and hypothyroid subjects

During the 7-day period of administration of 100 μg of LT_3 to two men, and one week after cessation of treatment in one man (J.P.), there were increases in phospholipid arachidonate (representing 2-6% of the total phospholipid fatty acids). The phospholipid linoleate decreased by approximately the same amount (Appendix Table 1).

Following thyroid hormone therapy, the hypothyroid patients showed, on average, a significant decrease in phospholipid concentrations ($P < 0.02$). These changes were associated with increases in phospholipid arachidonate ($P < 0.01$) and decreases in phospholipid oleate ($P < 0.025$ Table 18, Appendix Table 2).

(ii) Antithyroid therapy in thyrotoxic subjects

The treatment resulted in significant increases in serum phospholipid concentrations ($P < 0.01$) associated with increases in phospholipid linoleate ($P < 0.001$ Table 18).

(iii) Diseases of the thyroid

The concentration of serum phospholipids were found to be nearly twice as high in patients with **myxoedema** compared to those with **thyrotoxicosis** The percentage of phospholipid

TABLE 18.

Changes in percentage composition of serum phospholipid fatty acids in response to factors influencing fatty acid mobilisation.

Factors influencing fatty acid mobilisation	No. of subjects	No. of observations	Serum FFA concentration µEq/l			Serum phospholipid concentration mg/100 ml.			Serum phospholipid fatty acid composition (%)													
									16:0*			18:0*			18:1*			18:2*			20:4 *		
			Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference	Before treatment	After treatment	Observed difference
Thyroid hormone therapy in hypothyroid subjects (average fasting values in treated compared with untreated state)	9	16	751 (±337)	947 (±326)	+196[a] (± 185)	369 (±74)	271 (± 63)	-98[c] (±73)	33 (±3.2)	33 (±4.2)	+0.2 (±2.2)	15 (±2.3)	15 (±2.2)	-0.2 (±1.5)	18 (±1.9)	16 (±1.8)	-1.9 (±2.0)	21 (±3.6)	19 (±3.3)	-1.5 (±1.4)	9 (±1.8)	11 (±1.4)	+1.6 (±1.4)
					P<0.05			P<0.02									P<0.025						P<0.01
Antithyroid therapy in thyrotoxic subjects (average fasting values in treated compared with untreated state)	7	11	952 (±195)	634 (±130)	-318[b] (±191)	196 (±37)	299 (± 59)	+103[d] (±28)	30 (±3.8)	29 (±1.7)	-1.4 (±3.6)	17 (±2.0)	16 (±0.7)	-0.9 (±1.1)	16 (±1.4)	16 (±1.0)	-0.5 (±1.3)	19 (±3.4)	23 (±1.2)	+4.1 (±1.8)	13 (±1.8)	12 (±1.2)	-0.8 (±2.6)
					P<0.01			P<0.01												P<0.001			
HGH injection (average fasting values observed between the 1st and 3rd day of HGH administration compared with the average fasting values in the pre-treatment period)	7	17	612 (±195)	671 (±111)	+59 (±74)	278 (±41)	209 (± 35)	-69[e] (±42)	32 (±3.8)	33 (±4.1)	+1.0 (±3.2)	16 (±1.5)	15 (±2.0)	-0.6 (±1.2)	20 (±2.2)	18 (±2.0)	-2.3 (±0.7)	18 (±2.1)	20 (±3.4)	+1.5 (±1.7)	11 (±1.1)	12 (±1.6)	+0.5 (±1.2)
								P<0.025									P<0.001						
Noradrenaline infusion (changes observed after a 15-minute noradrenaline infusion	6	6	535 (±132)	846 (±100)	+311 (±222)	293 (±38)	260 (± 65)	-33[f] (±24)	35 (±4.3)	30 (±4.6)	-4.3 (±1.4)	16 (±0.8)	16 (±0.9)	-0.2 (±1.0)	17 (±2.0)	17 (±1.8)	-0.3 (±1.3)	20 (±1.9)	19 (±3.1)	-1.0 (±2.6)	8 (±2.3)	12 (±3.3)	+3.7 (±1.3)
					P<0.02					P<0.001													P<0.005
Fasting (changes observed after prolongation of overnight fast by 8 hours)	12	12	688 (±239)	976 (±269)	+288 (±275)	295 (±50)	295 (± 79)	0.0[g] (±29)	30 (±5.1)	33 (±4.0)	+2.8 (±4.1)	16 (±1.6)	15 (±1.5)	-1.0 (±1.6)	17 (±3.9)	16 (±3.4)	-0.6 (±1.5)	22 (±4.2)	19 (±4.0)	-2.5 (±2.1)	11 (±1.6)	11 (±2.1)	0.0 (±1.2)
					P<0.005					P<0.05										P<0.005			

[a] Serum FFA concentrations were estimated in 7 subjects

[b] Serum FFA concentrations were estimated in 6 subjects

[c] Serum phospholipid concentrations were estimated in 6 subjects

[d] Serum phospholipid concentrations were estimated in 4 subjects

[e] Serum phospholipid concentrations were estimated in 4 subjects

[f] Serum phospholipid concentrations were estimated in 4 subjects

[g] Serum phospholipid concentrations were estimated in 6 subjects

* Fatty acid short hand designation.

TABLE 19.

The percentage composition of serum phospholipid fatty acids in normal healthy subjects, in subjects with ischaemic heart disease and in subjects with different hormonal disorders.

No.	Group of subjects	No. of subjects	Serum phospholipid concentration mg/100 ml. Mean SD	Serum phospholipid fatty acid composition (%) 16:0* Mean SD	18:0* Mean SD	18:1* Mean SD	18:2* Mean SD	20:4* Mean SD
No. 1a	Normal males aged 26-51	12	230 ± 25[a]	28 ± 4.3	16 ± 1.5	16 ± 2.3	25 ± 3.3	11 ± 1.6
No. 1b	Normal females aged 50-60	44	n.e.	31 ± 1.8	17 ± 0.6	16 ± 3.8	21 ± 2.8	11 ± 1.8
No. 2**	IHD chronic, males aged 33-51	23	286 ± 21[b]	31 ± 4.7	15 ± 1.3	16 ± 2.0	22 ± 4.7	11 ± 2.3
No. 2a	" with higher serum phospholipid conc.	8	367 ± 80	37 ± 2.8	15 ± 1.3	16 ± 1.9	19 ± 2.6	10 ± 2.1
No. 2b	" with higher serum FFA conc. (760 ± 148 µEq/l)	10	275 ± 68[c]	33 ± 5.1	16 ± 1.0	17 ± 1.6	22 ± 1.3	10 ± 1.8
No. 1a - No. 2			P < 0.001					
No. 1a - No. 2a			P < 0.001	P < 0.001			P < 0.001	
No. 1a - No. 2b				P < 0.025			P < 0.02	
No. 3	IHD acute, post myoc. infarction 24-32 hrs. 7 males, 1 female aged 45-62	8	220 ± 136	37 ± 2.8	14 ± 1.4	17 ± 2.0	18 ± 2.5	10 ± 2.6
No. 1a - No. 3				P < 0.001			P < 0.001	
No. 4	Hyperthyroid, 3 males, 2 females, aged 50-60 3 females aged 20-30	8	196 ± 37[d]	30 ± 3.7	17 ± 1.4	16 ± 1.4	19 ± 3.5	13 ± 1.8
No. 5	Hypothyroid, 1 male 8 females, aged 50-60	9	369 ± 74[e]	33 ± 3.4	15 ± 2.7	18 ± 2.1	21 ± 3.6	9 ± 1.8
No. 1a- No. 4								P < 0.02
No. 1b - No. 4								P < 0.02
No. 1b - No. 5								P < 0.05
No. 4 - No. 5								P < 0.005
No. 6	Acromegaly, 3 females aged 50-60	3	233[f]	32 ± 0.7	16 ± 1.4	18 ± 3.3	21 ± 3.2	8 ± 1.6

* Fatty acid short hand designation.

** Patients with angina pectoris or 2 months-2 years after myocardial infarction.

The P values in frames were calculated from groups of subjects of similar age and the same sex.

[a] Serum phospholipid concentrations were estimated in 6 subjects.

[b] Serum phospholipid concentrations were estimated in 10 subjects.

[c] Serum phospholipid concentrations were estimated in 7 subjects.

[d] Serum phospholipid concentrations were estimated in 4 subjects.

[e] Serum phospholipid concentrations were estimated in 6 subjects.

[f] Serum phospholipid concentrations were estimated in 1 subject.

arachidonate was approximately 50% higher in hyperthyroid than in hypothyroid patients (Table 19).

As compared to the normal male or female groups, hyperthyroid patients had somewhat higher, and hypothyroid patients somewhat lower percentages of phospholipid arachidonate, (Fig. 12).

Human Growth Hormone

(i) Effects produced within hours after injection of HGH

It was noted that, during the time of acute mobilisation of serum FFA, e.g., 4 hours after the administration of HGH to fasting men, there was a short-lived decrease in phospholipid concentrations, which had returned to normal 8 hours after treatment. These changes were accompanied by somewhat higher percentages of phospholipid palmitate in all three men under observation. (Appendix Table 6a).

(ii) The effects of daily injections of HGH

Serum phospholipid concentrations were investigated in only 5 out of the 7 subjects under study (Appendix Tables 5 and 6b). During the daily administration of HGH, considerable decreases in serum phospholipid concentration were observed in 4 out of the 5 subjects. On average, in the group studied, phospholipid concentrations were significantly decreased during the daily administration of HGH ($P < 0.025$, Table 18). Also, during

Fig. 12 Changes in percentages of cholesteryl and phospholipid arachidonate and linoleate in relation to thyroid function and in response to treatment with thyroid hormones, antithyroid therapy and noradrenaline infusion (Mean ± 1 S.D.)


hypothyroid subjects


euthyroid subjects

" " (middleaged males)

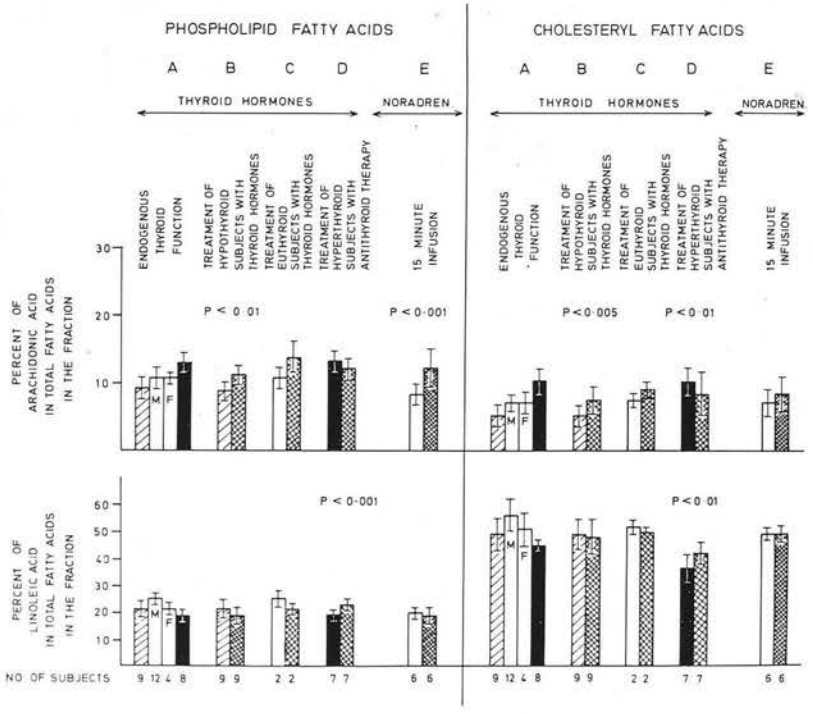
" " (postmenopausal females)


hyperthyroid subjects


after treatment

B, C and E changes accompanied by increases of serum FFA concentrations

D changes accompanied by decreases of serum FFA concentrations


PHOSPHOLIPID FATTY ACIDS
CHOLESTERYL FATTY ACIDS
A B C D E
THYROID HORMONES
NORADREN.
ENDOGENOUS THYROID FUNCTION
TREATMENT OF HYPOTHYROID SUBJECTS WITH THYROID HORMONES
TREATMENT OF EUTHYROID SUBJECTS WITH THYROID HORMONES
TREATMENT OF HYPERTHYROID SUBJECTS WITH ANTITHYROID THERAPY
15 MINUTE INFUSION
PERCENT OF ARACHIDONIC ACID IN TOTAL FATTY ACIDS IN THE FRACTION
P < 0·01
P < 0·001
P < 0·005
P < 0·01
PERCENT OF LINOLEIC ACID IN TOTAL FATTY ACIDS IN THE FRACTION
P < 0·001
P < 0·01
NO. OF SUBJECTS
9 12 4 8
9 9
2 2
7 7
6 6
9 12 4 8
9 9
2 2
7 7
6 6

the days following the cessation of HGH administration, these patients continued to exhibit reduced concentrations of serum phospholipids (Appendix Table 5).

Compared with the large observed changes in total serum phospholipid concentrations, changes in phospholipid fatty acids were small. Of statistical significance were the decreases in phospholipid oleate ($P < 0.001$) observed during the period of HGH administration.(**Appendix Table 5,6b , Table 18**)

(iii) Acromegaly

No specific changes in serum phospholipid concentrations or in the composition of phospholipid fatty acids were observed in these patients (Table 19).

Noradrenaline

15 minutes after the commencement of the infusion of noradrenaline, no significant changes in phospholipid concentrations were observed but a significant alteration was noted in phospholipid fatty acid composition (Table 18).

Phospholipid arachidonate increased in all patients under observation by an average of 50% above the original value, while phospholipid palmitate decreased. These changes are statistically significant ($P < 0.005$, $P < 0.001$, respectively).

Fasting

When the overnight fast was prolonged by 8 hours, no change of statistical significance occurred in serum phospholipid concentrations, but the phospholipid fatty acid composition was

significantly altered (Table 18, Appendix Table 9). The phospholipid linoleate decreased ($P < 0.005$) and the phospholipid palmitate increased ($P < 0.05$).

Ischaemic heart disease

(i) Acute myocardial infarction

Twentyfour to 32 hours after acute myocardial infarction levels of serum phospholipids were variable (Appendix Table 10). The most significant difference in the phospholipid fatty acid composition of this group as compared to either the normal male or female group, was the higher observed percentage of phospholipid palmitate ($P < 0.001$) and lower percentage of phospholipid linoleate ($P < 0.05$, Table 19). With respect to the average percentages of individual phospholipid fatty acids, there is a similarity in the fatty acid composition of these patients with the hyperphospholipidaemic ones.

(ii) Chronic ischaemic heart disease

When the entire group of 23 male subjects was compared with the normal group of healthy male subjects, no significant differences were found to exist in the phospholipid fatty acids. However, significant elevation of phospholipid palmitate was noted in two selected groups of diseased patients, one with high serum phospholipid concentrations and the second with high serum FFA concentrations (Table 19), and these also had significantly lower phospholipid linoleate percentages.

CHANGES IN THE COMPOSITION OF SERUM CHOLESTERYL FATTY ACIDS IN RESPONSE TO DIFFERENT FACTORS INFLUENCING FATTY ACID MOBILISATION

Thyroid hormone

(i) Thyroid hormone treatment in normal and hypothyroid subjects

In the two normal men to whom 100 μg of LT_3 was given on each of the 7 days, it was observed that the serum cholesterol concentrations during the week of administration of LT_3, and during the following week, decreased slightly but steadily. Cholesteryl arachidonate was elevated by treatment, but since only two subjects were under study, no significance could be proved statistically for these increases (Appendix Table 1).

In the hypothyroid subjects, serum cholesterol concentrations were significantly decreased as a result of treatment with thyroid hormone (Table 20). The cholesteryl arachidonate was significantly increased ($P < 0.005$). Two patients who were studied under balanced dietetic conditions showed, in addition, decreases in cholesteryl linoleate (Appendix Table 2).

(ii) Antithyroid therapy in thyrotoxic subjects

Thyrotoxic subjects treated with antithyroid therapy showed significant changes in serum cholesterol concentrations and in the composition of all major fatty acid components (Table 20, Appendix Tables 3 and 4). The increases in serum cholesterol concentrations ($P < 0.005$) were accompanied by increased proportions of cholesteryl linoleate ($P < 0.01$), and decreased proportions of cholesteryl palmitate ($P < 0.02$), oleate

TABLE 20.

Changes in percentage composition of serum cholesteryl fatty acids in response to factors influencing fatty acid mobilisation.

Factors influencing fatty acid mobilisation	No. of subjects	No. of observations	Serum FFA concentration µEq/l. Before treatment	After treatment	Observed difference	Serum cholesterol concentration mg/100 ml. Before treatment	After treatment	Observed difference	Serum cholesteryl fatty acid composition (%) 16:0 * Before treatment	After treatment	Observed difference	18:1 * Before treatment	After treatment	Observed difference	18:2 * Before treatment	After treatment	Observed difference	20:4 * Before treatment	After treatment	Observed difference
Thyroid hormone therapy in hypothyroid subjects (average fasting values in treated compared with untreated state)	9	16	751 (±337)	947 (±326)	+196[a] (±185) P<0.05	364 (±79)	251 (± 56)	-113 (±69) P<0.005	12 (±1.9)	12 (±1.4)	-0.2 (±2.2)	26 (±2.9)	25 (±3.3)	-1.2 (±0.8)	49 (±5.8)	48 (±6.7)	-1.2 (±4.8)	5 (±1.4)	7 (±2.1)	+1.9 (±1.4) P<0.005
Antithyroid therapy in thyrotoxic subjects (average values in treated compared with untreated state)	7	11	952 (±195)	634 (±130)	-318[b] (±191) P<0.01	159 (±28)	231 (± 23)	+72 (±38) P<0.005	12 (±0.9)	10 (±1.7)	-1.7 (±1.3) P<0.02	25 (±2.8)	23 (±3.3)	-1.9 (±1.7) P<0.05	46 (±3.1)	52 (±4.0)	+5.9 (±3.9) P<0.01	10 (±2.1)	8 (±2.9)	-2.0 (±1.3) P<0.01
HGH injection (average fasting values observed between the 1st and 3rd day of HGH administration compared with the average fasting values in the pretreatment period)	7	16	612 (±195)	671 (±111)	+59 (±74)	225 (±53)	222 (± 42)	-3 (±28)	12 (±0.3)	15 (±4.6)	+3.1 (±4.1)	25 (±3.0)	28 (±4.0)	+3.4 (±3.4) P<0.05	44 (±1.6)	38 (±7.4)	-5.9 (±6.2) P<0.05	7 (±1.3)	6 (±2.2)	-0.8 (±0.9)
Noradrenaline infusion (changes observed after a 15-minute noradrenaline infusion)	6	6	535 (±132)	846 (±100)	+311 (±222) P<0.02	306 (±130)	294 (±129)	-12 (±1.1)	12 (±1.0)	12 (±1.4)	+0.2 (±1.0)	27 (±3.8)	27 (±2.9)	0.0 (±2.0)	48 (±2.4)	48 (±2.8)	+0.2 (±2.9)	7 (±2.1)	8 (±2.9)	+1.3 (±1.3)
Fasting (changes observed after prolongation of overnight fast by 8 hours)	12	12	688 (±239)	976 (±269)	+288 (±275) P<0.005	253 (±81)	230 (± 81)	-23 (±22)	11 (±2.5)	10 (±1.8)	-0.8 (±1.5)	25 (±5.5)	25 (±5.5)	+0.2 (±1.2)	50 (±8.8)	50 (±7.7)	+0.4 (±2.5)	6 (±1.2)	7 (±2.9)	+0.8 (±1.6)

[a] Serum FFA concentrations were estimated in 7 subjects

[b] Serum FFA concentrations were estimated in 6 subjects

* Fatty acid short hand designation.

TABLE 21.

The percentage composition of serum cholesteryl fatty acids in normal healthy subjects, in subjects with ischaemic heart disease and in subjects with different hormonal disorders

No.	Group of subjects	No. of subjects	Serum cholesterol concentration mg/100 ml.		Serum cholesteryl fatty acid composition (%)							
					16:0*		18:1*		18:2*		20:4*	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
No. 1a	Normal males aged 26-51	12	197	± 25	10	± 1.2	20	± 3.3	56	± 5.7	7	± 1.4
No. 1b	Normal females aged 50-60	4	263	± 38	12	± 1.0	23	± 3.9	51	± 7.5	7	± 1.4
No. 2**	IHD chronic, males aged 33-51	23	287	± 22	12	± 2.5	23	± 3.1	50	± 6.2	8	± 2.3
No. 2a	" with higher cholesterol conc.	10	335	± 42	12	± 3.4	24	± 3.7	50	± 2.2	9	± 3.0
No. 2b	" with higher FFA conc. (760 ± 148 µEq/l.)	10	286	± 35	12	± 1.5	24	± 2.2	50	± 4.6	7	± 1.4
No. 1a - No. 2			P < 0.001		P < 0.01		P < 0.02		P < 0.01			
No. 1a - No. 2a			P < 0.001				P < 0.02		P < 0.01			
No. 1a - No. 2b							P < 0.005		P < 0.02			
No. 3	IHD, acute, post myoc. infarction 24-32 hrs. 7 males, 1 female, aged 45-62	8	224	± 32	12	± 1.1	27	± 2.5	48	± 3.4	7	± 4.7
No. 1a - No. 3							P < 0.001		P < 0.005			
No. 4	Hyperthyroid, 3 males, 2 females aged 50-60; 3 females aged 20-30	8	157	± 64	12	± 1.0	25	± 3.6	45	± 2.8	10	± 2.0
No. 5	Hypothyroid, 1 male, 8 females aged 50-60	9	365	± 24	12	± 2.6	26	± 3.1	49	± 5.5	5	± 1.4
No. 1a - No. 4							P < 0.01		P < 0.001			
No. 6	Acromegaly females aged 50-60	3	223	± 38	12	± 1.0	25	± 5.0	50	± 6.6	5	± 1.6

* Fatty acid shorthand designation.

** Patients with angina pectoris or 2 months-2 years after myocardial infarction.

The P values in frames were calculated from groups of subjects of similar age and the same sex

($P < 0.05$) and arachidonate ($P < 0.01$).

(iii) Diseases of the thyroid

The average levels of serum cholesterol were more than twice as high in the hypo- as in the hyperthyroid patients. The greatest differences in the fatty acid spectrum of the cholesterol esters were between the levels of cholesteryl arachidonate, which were, on average, twice as high in hyper- as in hypothyroid patients (Table 21, Fig. 12). As compared to the normal male or female groups, hyperthyroid patients had somewhat higher, and hypothyroid patients somewhat lower percentages of cholesteryl arachidonate.

Human growth hormone

(i) Effects produced within hours after the injection

No significant changes were observed in either the cholesterol concentration or the cholesteryl fatty acid composition during fasting, 4 and 8 hours after HGH injections (Appendix Table 6a).

(ii) The effects of daily injections of HGH

There were no significant changes in the fasting serum cholesterol concentrations during the period of HGH administration (Appendix Tables 5 and 6b). When the fasting results from all subjects during the period of HGH administration were compared with those (also fasting) obtained during the pretreatment period (Table 20), it was found that the cholesteryl oleate had significantly increased ($P < 0.05$) and the cholesteryl linoleate had significantly decreased ($P < 0.05$).

(iii) Acromegaly

There were no differences to be observed either in levels of cholesterol or in the cholesteryl fatty acids (Table 21).

Noradrenaline

No significant changes were observed either in the concentration or in fatty acid composition of cholesteryl esters during the noradrenaline infusion. In 3 out of the 6 men under observation, there were small increases in cholesteryl arachidonate (Appendix Table 8).

Fasting

When fasting was prolonged by 8 hours, there were no significant changes either in serum cholesterol levels or in cholesteryl fatty acid composition (Table 20, Appendix Table 9).

Ischaemic heart disease (IHD)

(i) Acute myocardial infarction

The serum cholesterol concentrations of this group did not differ from those of the normal healthy group. The fatty acid composition, however, was significantly altered (Table 21, Appendix Table 10). Decreases in cholesteryl linoleate and increases in cholesteryl oleate were statistically significant ($P < 0.005$ and $P < 0.001$ respectively). It has been observed that the described changes increased between the first and second, or second and third days after the onset of pain. Cholesteryl linoleate was lowered and cholesteryl oleate was increased during these days in 6 out of the 8 subjects under

study (A.B., J.G., J.H., J.Cl., J.C., J.S.; Appendix Table 10).

(ii) Chronic ischaemic heart disease

As compared to the normal healthy male group, patients with ischaemic heart disease had significantly higher levels of serum cholesterol. Cholesteryl fatty acids were characterised by significantly increased percentages of cholesteryl palmitate ($P < 0.01$) and oleate ($P < 0.02$), and by decreased percentages of cholesteryl linoleate ($P < 0.01$; Table 21).

When, from the entire group of 23 male patients with IHD, subjects were selected according to the levels of serum FFA or serum cholesterol, the smaller groups with hypercholesterolaemia or high serum FFA levels did not differ significantly from the entire group. However, in the group with high serum FFA levels the statistical significance of increases in oleic acid percentages reached a higher level (Table 21).

INTERRELATIONSHIP BETWEEN THE CONCENTRATIONS OF SERUM FFA AND PERCENTAGES OF FFA MONOUNSATURATED ACIDS

Since it has been observed that increases in serum FFA concentrations were generally associated with increased percentages of FFA oleic acid, and in some cases also palmitoleic acid, the coefficient of correlation was calculated

between the sum of percentages of these two acids and between the serum FFA concentrations in all estimations performed. A highly significant correlation was obtained. The coefficient of correlation r = 0.64, was 7 times higher than its standard error: $1/\sqrt{n-1} = 0.084$.

No significant correlation, however, was obtained when the coefficient of correlation was calculated for levels of serum FFA ranging from 500 to 1000 μEq/l. The scatter diagram (Fig. 13) shows that, although high levels of serum FFA are usually accompanied by higher percentages of monounsaturated acids (44 - 62), within the middle range the points show a wide variation.

THE DEGREE OF DIFFERENCE BETWEEN PERCENTAGES OF FFA OLEIC ACID AND PERCENTAGES OF TRIGLYCERIDE OLEIC ACID

The mean values of oleic acid percentages in the free and in the triglyceride fatty acids are illustrated by histograms in Fig. 14. It is shown that the magnitude of the difference between the mean percentages of oleic acid in the FFA and triglyceride fractions were about the same in healthy males as in hypothyroid patients. The difference between the percentage level of FFA and triglyceride oleic acid shown in thyrotoxic patients is very small, and nearly nonexistent in patients after acute myocardial infarction. The treatment of hypothyroid

Fig. 13 Correlation between the serum FFA concentrations and between the percentages of FFA monounsaturated acids

Monounsaturated acids; oleic and palmitoleic acids

The coefficient of correlation $r = 0.64$, its standard error, $1/\sqrt{n-1} = 0.084$, $n = 136$

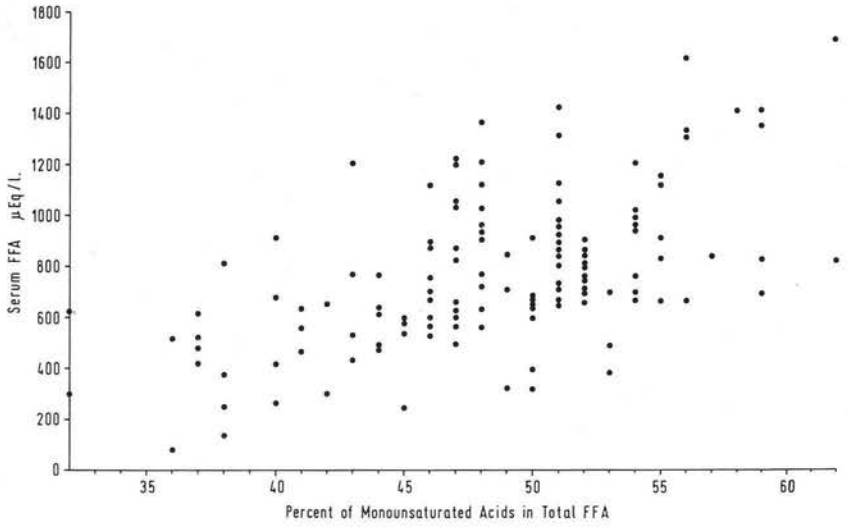

1800
1600
1400
1200
1000
800
600
400
200
0
Serum FFA µEq/l.
35
40
45
50
55
60
Percent of Monounsaturated Acids in Total FFA

Fig. 14 Percentages of FFA and triglyceride oleic acid in relation to thyroid status, acromegaly and ischaemic heart disease

Percentage of FFA oleic acid

Percentage of triglyceride oleic acid

PERCENT OF OLEIC ACID IN TOTAL FATTY ACIDS IN THE FRACTION
60
50
40
30
20
10
NORMAL (MALES)
NORMAL (FEMALES)
HYPOTHYROID STATE
HYPOTHYROID STATE – TREATED
HYPERTHYROID STATE
HYPERTHYROID STATE – TREATED
ISCHAEMIC HEART DISEASE (CHRONIC)
MYOCARDIAL INFARCTION (ACUTE)
ACROMEGALY
SERUM FFA CONCENTRATION. (μEq./l.)
490 (± 34)
620 (± 125)
751 (± 337)
947 (± 74)
887 (± 115)
676 (± 112)
618 (± 202)
804 (± 120)
386 (± 186)

patients with thyroid hormones led to a decrease, and antithyroid therapy given to thyrotoxic patients to an increase, in the degree of these differences.

THE ASSOCIATION OF INCREASES IN FFA CONCENTRATION WITH INCREASE IN OLEIC ACID PERCENTAGES IN THE TRIGLYCERIDE AND IN THE CHOLESTERYL FATTY ACIDS

It has been observed that changes in the fasting concentrations of serum FFA during antithyroid therapy in thyrotoxic patients (in all subjects) and during thyroid hormone therapy in hypothyroid and normal subjects (only those on the balanced diet) were accompanied by changes in oleic acid percentages, not only in the FFA fraction but also in the triglyceride and cholesteryl fractions. Usually changes in percentages of cholesteryl and triglyceride oleate were directly related to the changes in serum FFA, while corresponding changes in the percentages of linoleic acid were inversely related to the changes in serum FFA concentrations (Figs. 15 and 16).

Similarly, it has already been demonstrated that cholesteryl oleate increased between the second and the third day of HGH administration in most persons who showed elevated levels of serum FFA 24 hours after the first injection (Fig. 17). The changes in percentages of cholesteryl oleate were accompanied by reciprocal changes in percentages of cholesteryl linoleate. The daily administration of HGH did not, however,

Fig. 15 Changes in FFA concentrations and corresponding changes in oleic and linoleic acid percentages in lipid fractions in response to the treatment with thyroid hormones

Increases are shown over the line in white bars

Decreases are shown under the line in black bars

Abbreviations:

TG - triglycerides
PL - phospholipids
CE - cholesterol esters
N.E. - Not estimated

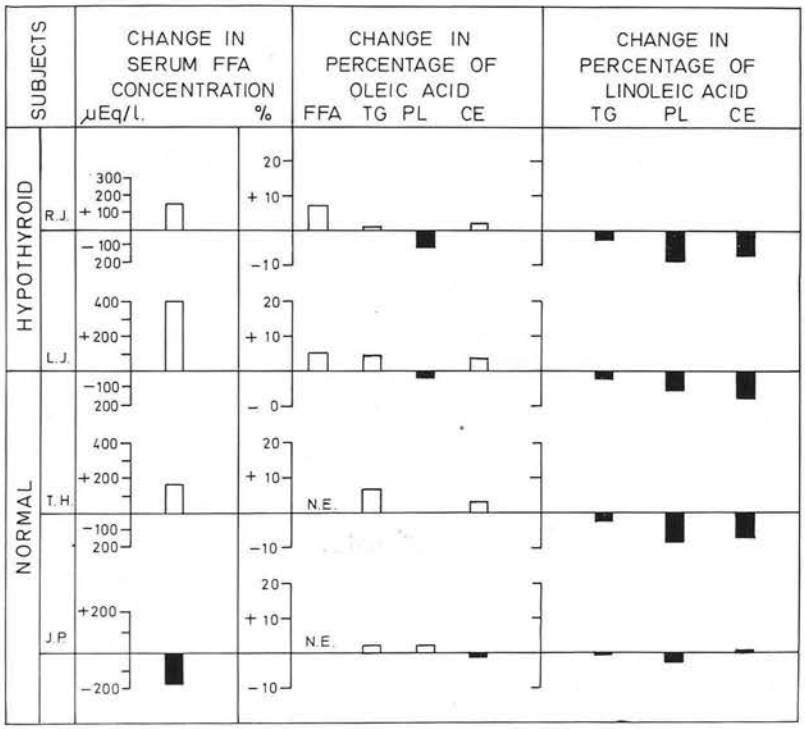

SUBJECTS
CHANGE IN SERUM FFA CONCENTRATION
μEq/l.
%
CHANGE IN PERCENTAGE OF OLEIC ACID
FFA TG PL CE
CHANGE IN PERCENTAGE OF LINOLEIC ACID
TG PL CE
HYPOTHYROID
NORMAL
R.J.
L.J.
T.H.
J.P.
300
200
+ 100
− 100
200
400
+200
−100
200
+200
−200
20
+ 10
−10
20
+ 10
− 0
20
+ 10
N.E.
−10
20
+ 10
N.E.
−10

Fig. 16 Changes in FFA concentrations and corresponding changes in oleic and linoleic acid percentages in lipid fractions in response to antithyroid therapy

Increases are shown over the line in white bars

Decreases are shown under the line in black bars

Abbreviations :

TG - triglycerides
PL - phospholipids
CE - cholesterol esters
N.E. - Not estimated

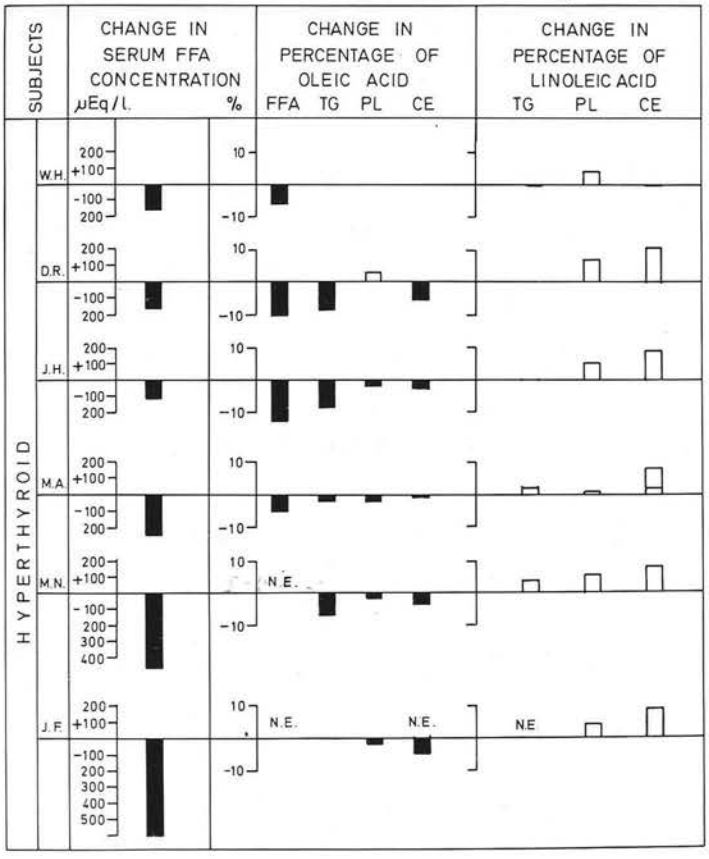

SUBJECTS
CHANGE IN SERUM FFA CONCENTRATION
µEq/l.
%
CHANGE IN PERCENTAGE OF OLEIC ACID
FFA TG PL CE
CHANGE IN PERCENTAGE OF LINOLEIC ACID
TG PL CE
HYPERTHYROID
W.H.
D.R.
J.H.
M.A.
M.N.
J.F.
200
+100
-100
200
300
400
500
10
-10
N.E.

Fig. 17 Changes in FFA concentrations and subsequent changes in oleic and linoleic acid percentages in lipid fractions in response to HGH daily injections

Changes in FFA concentration = after 1 day of treatment (24 hours after first injection

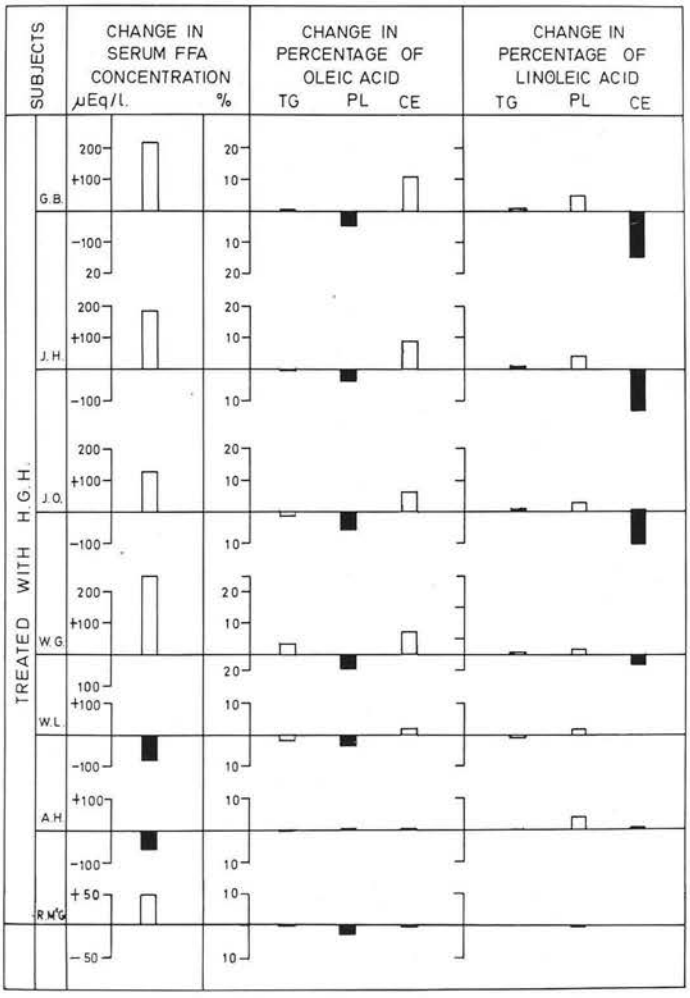
Changes in oleic and linoleic acid percentages = mean values from second and third day of treatment

Increases are shown over the line in white bars

Decreases are shown under the line in black bars

Abbreviations :

TG - triglycerides
PL - phospholipids
CE - cholesterol esters


SUBJECTS
CHANGE IN SERUM FFA CONCENTRATION
μEq/l.
%
CHANGE IN PERCENTAGE OF OLEIC ACID
TG PL CE
CHANGE IN PERCENTAGE OF LINOLEIC ACID
TG PL CE
TREATED WITH H.G.H.
G.B.
J.H.
J.O.
W.G.
W.L.
A.H.
R.M.G.
200
+100
−100
20
100
+50
−50
10

influence the percentages of triglyceride oleate.

Similarly, when compared to normal persons, patients in the days following myocardial infarction showed increases in cholesteryl and triglyceride oleate (Table 21,17)and these changes were intensified between the first and second, or second and third day after the onset of the acute event (Appendix Table 10). In these circumstances also a reciprocal relationship of changes in the percentages of cholesteryl (but not triglyceride) linoleate was observed.

DISCUSSION

THE CONCENTRATION OF SERUM LIPIDS IN RELATION TO THYROID HORMONE

Thyroid hormone therapy in hypothyroid subjects induced significant increases in serum FFA, and significant decreases in serum triglyceride, phospholipid and cholesterol concentrations. Antithyroid therapy in thyrotoxic subjects induced significant changes in the opposite direction. These observations have not previously been reported in similar studies, but could be expected from the following evidence.

Rich et al., (1959) reported that endogenous or induced thyrotoxicosis is accompanied by increases in the levels of fasting serum FFA. The authors observed also an increase in the levels of serum FFA 6 hours after the injection of L-triiodothyronine. Harlan et al., (1963) showed that the fasting levels of serum FFA and mobilisation after catecholamine stimulation are dependent upon the clinical status of thyroid function. Feldman and Carter (1963) reported that treatment with sodium-D-thyroxine in subjects with hyperlipidaemia was effective in lowering serum triglycerides and total cholesterol. Along the same lines, Moses and Danowski (1963) have reported decreases in serum cholesterol (both α and β-lipoprotein) and phospholipids (total and β-lipoprotein) after the treatment of normal subjects with desiccated thyroid. The last-named authors

did not find alterations in serum triglyceride and FFA concentrations; however, in other experiments carried out on hyperlipidaemic patients the serum triglycerides were found to be reduced (Moses et al., 1964).

The present results do not support the suggestion of Harlan et al., (1963) that the hypothyroid state is accompanied by lower than normal resting levels of serum FFA. The average level of serum FFA in myxoedematous patients was rather high. The results in individual subjects obtained on various occasions suggest wide variability in serum FFA levels in these patients. After treatment with thyroid hormones, the serum FFA levels in these patients reached the levels observed in hyperthyroid subjects, although treatment led to the euthyroid state. These results indicate an increased rather than a reduced ability for FFA mobilisation in these patients.

THE CONCENTRATION OF SERUM LIPIDS IN RELATION TO HUMAN GROWTH HORMONE (HGH)

Human growth hormone injections administered to fasting men induced increases in serum FFA levels within hours. This had been reported previously by Raben and Hollenberg (1959) who described changes only up to 4 hours and did not establish the time of maximal effect. In the present study, the increases were greater at 8 than at 4 hours after the injection, but the time

of maximal increase was again not established, since estimations were not performed between 4 and 8 hours, nor at longer intervals. Raben and Hollenberg reported that serum FFA levels returned to normal 24 hours after HGH administration. In the present study, 4 out of the 7 subjects had higher levels of serum FFA 24 hours after the injection than before. Rises were observed after the first and second, but not after the third injection. Raben and Hollenberg reported also that food completely abolished the effects of HGH. The present study showed that all daily levels were raised during the first two days of HGH injections, in spite of daily meals. The greatest rise was observed 7 hours after HGH injection (approximately 4 hours after the second meal).

The difference between the present observations and those of Raben and Hollenberg may be due, in part, to the larger doses of HGH used in this experiment (25 mg against 3 - 8 mg).

Recently, Landon et al., (1966) suggested that abnormally high fasting levels of serum FFA occur in patients with acromegaly. The present findings do not confirm this observation, and in fact levels of serum FFA lower than normal were noted in two out of the three acromegalic women under study.

There are no reported studies on the effects of HGH on the concentration of esterified lipids in man. The present study indicates the effectiveness of HGH injections in lowering serum phospholipid concentrations.

STUDIES ON FREE FATTY ACIDS (FFA)

(i) Similarities and differences in the effects of various factors inducing FFA mobilisation

The results presented here have demonstrated that many of the changes in the composition of serum FFA induced by different mobilising influences have common characteristics. Oleic acid was always the predominant participant in increases in serum FFA concentrations. The increase in serum FFA induced by thyroid therapy in hypothyroid patients was associated with a large increase in oleic acid, and the decrease in serum FFA induced by antithyroid therapy in thyrotoxic patients was associated with particularly low levels of oleic acid. Small increases in the percentage of oleic acid occurred when fasting was prolonged by 8 hours, and the increases were greatly intensified 8 hours after the administration of HGH under similar fasting conditions. Of the diseases studied, those with the highest levels of serum FFA (thyrotoxicosis and acute myocardial infarction) were accompanied by the highest percentages of serum free oleic acid.

These increases in the percentage of oleic acid were accompanied by a simultaneous decrease in percentages

of stearic and palmitic acid. The same inverse relationship holds after antithyroid therapy.

Changes induced by hormonal agents or by fasting have, to my knowledge, only been reported in animal studies. Feigelson et al., (1961) injected dogs with noradrenaline and observed increases in the percentages of oleic acid and decreases in stearic acid 8 hours after the injection. Norcia and Evans (1964) injected adrenaline into rabbits and, although there was no increase in total FFA in one of the groups of animals studied, there were increases in the percentages of oleic acid observed.

The literature provides little evidence concerning the composition of human serum fatty acids and, so far, the relationship between the increases in serum FFA concentrations or high fasting levels of serum FFA with high percentages of oleic acid and low percentages of saturated acids has not been stressed. One of the factors which is known to raise serum FFA concentrations is cigarette smoking. In one study, an increase in the proportions of free monounsaturated and a decrease in free saturated acids following smoking have been reported (Murchison and Fyfe, 1966), such as one might expect if the FFA rise were to be a noradrenaline-induced response. Along the same lines, changes have been reported in fatty acid composition during glucose tolerance tests (Soloff and Schwartz, 1966). When the data presented by these authors concerning the concentrations of individual serum

FFA were recalculated as percentages of total composition, it was observed that the decrease in FFA which followed $\frac{1}{2}$ hour to 3 hours after the glucose load was associated particularly with decreases in oleic acid percentages (with inverse changes in stearic acid), while the increases in FFA which followed 5 hours after a glucose load, and which exceeded the original values, were accompanied by increases in oleic acid percentages (with inverse changes in stearic acid). Evidence concerning the composition of serum FFA in atherosclerosis also supports the observations presented here. According to the data of Böttcher and Woodford (1961), patients with aortic atherosclerosis had higher percentages of oleic acid in the FFA of lipids extracted from protein fraction IV - V separated by Cohn's method. The data presented by Soloff and Schwartz (1966) on the fasting concentrations of total and individual serum FFA in normal subjects and patients convalescing from myocardial infarction show (after being recalculated as percentages of composition) that compared with normal controls ischaemic patients had higher concentrations of serum FFA and higher percentages of FFA oleic acid. These increases in oleic acid percentages were higher in the ischaemic patients with a diabetic-like response than those with a normal response to glucose tolerance tests. In another report, Schrade et al., (1963) stated that very large increases in free oleic acid were

associated with very high levels of serum FFA in diabetic coma.

The direct relationship between concentrations of FFA and percentage composition does not, however, always hold. Caren and Corbo (1966) have reported greatly elevated percentages of free oleic acid in pancreatic cystic fibrosis, a condition not associated with elevated fasting levels of serum FFA.

(ii) Interpretation of results

(a) Stimulation of FFA release from adipose tissue

There is overwhelming evidence to support the view that, in the postabsorptive state, plasma FFA derive exclusively from adipose tissue (Dole, 1956; Gordon and Cherkes, 1956; Gordon, 1957; Fredrickson and Gordon, 1958).

Fatty acid mobilisation from adipose tissue is known to be under neural and hormonal influences. Several reviews on this subject have been published (Wertheimer and Shafrir, 1960; Steinberg, 1963; Rudman, 1963; Randle, 1964; Chalmers, 1964; Carlson et al., 1965). Steinberg (1963) has cited eleven different hormones which have been shown in vitro and in vivo to have lipolytic activities.

Carlson et al., (1965) stated, "Hormonal factors may act on adipose tissue by stimulation of the lipolytic process, for example, catecholamine and glucagon, or by promoting glucose entry into adipose tissue cells as in the case of insulin" and

"The mode of action of the sympathetic nervous system in lipid mobilisation is perhaps by control of norepinephrine release which stimulates the lipolytic activity in adipose tissue".

The evidence that adrenaline stimulates lipolysis of triglycerides in adipose tissue comes from numerous experiments in vitro (Leboeuf et al., 1959; Lynn et al., 1959; Vaughan and Steinberg, 1963) and in vivo (Dole, 1956; Gordon and Cherkes, 1956; Havel and Goldfien, 1959; Nestel, 1964). In the in vitro studies, the results with noradrenaline were quantitatively similar to those obtained with adrenaline (Vaughan and Steinberg, 1962; Vaughan and Steinberg, 1963). In the in vivo experiments, noradrenaline was found to elicit more sustained FFA elevation, but without the hyperglycemia (Goldfien and Havel, 1959) that was observed after adrenaline administration (Shafrir and Steinberg, 1960).

An indirect or "permissive" action has been ascribed to thyroid hormone. Debons and Schwartz (1961) found that fat pads from hypothyroid animals did not show increased release of FFA after addition of adrenaline to the incubation medium; on the other hand, the fat pads from hyperthyroid animals showed an exaggerated FFA release in response to adrenaline. The authors observed also that adipose tissue from animals treated with triiodothyronine released FFA at a higher rate

than normal, and showed an exaggerated response to adrenaline *in vitro*. Along the same lines, Deykin and Vaughan (1963) reported that adipose tissue from animals treated with triiodothyronine contained more FFA than tissue from euthyroid animals. Steinberg (1963) suggested that the effects of thyroid hormones may be due to a potentiation of the action of the endogenous catecholamines.

The mode of action of HGH is not known (Steinberg,1963; Chalmers, 1964). It produces lipolytic effects when administered *in vivo* (Raben and Hollenberg,1959), but *in vitro* high concentrations are required to produce any lipolytic effects (Raben, 1959). Vaughan and Steinberg (1963) suggested that HGH may not produce any lipolytic effects in *in vitro* experiments, and that the observed lipolytic action in these experiments may be attributed to trace contamination of the HGH preparation with TSH.

The prolongation of fasting also stimulates an adipokinetic response. Increases in serum FFA after the prolongation of fasting have been reported previously (Dole, 1956; Gordon, 1956; Recant *et al.*, 1963). It is not known what role hormones play in the mobilisation of fatty acids during fasting. Chalmers *et al.*, (1960) have reported that, during fasting or the restriction of carbohydrate intake, human

urine contains a potent fat mobilising substance (FMS). The FMS increases the release of FFA from adipose tissue in vitro. An intact pituitary gland is necessary for the appearance of FMS in the urine (Chalmers et al., 1960). The active principle has, however, not yet been obtained in pure form, and its chemical nature is unknown (Chalmers, 1964).

(b) Differential FFA release from adipose tissue

In normal healthy subjects, the fatty acid composition of serum FFA, and of adipose tissue triglycerides from which they are derived differ mainly in their relative proportions of oleic and stearic acids. Plasma FFA contains proportionally more stearic and less oleic acid than adipose tissue fatty acids (Cornwell et al., 1962). During the acute mobilisation of serum FFA (8 hours after noradrenaline injection given to dogs), the composition of plasma FFA resembles that of adipose tissue triglyceride fatty acids (Feigelson et al., 1961).

Many authors have tried to explain why the composition of plasma FFA normally differs from that of adipose tissue, since adipose tissue is the main source of plasma FFA. It has been suggested that the specificity of tissue lipases for certain ester linkages may be of importance in determining the pattern of mobilised FFA (Hollenberg and Douglas, 1962). The lipolytic enzymes catalyse hydrolysis of the ester linkage in the α-position of the triglyceride molecule, which is mainly

occupied by saturated acids. Mattson and Lutton (1958) have found that the specific localisation of fatty acids in the triglyceride molecule varies from species to species. In human, horse, and beef adipose tissue the α -positions carry mainly the saturated acids, while the β -position carries the unsaturated ones. In the rat and dog the distribution is more random.

Preferential release of saturated acids during the hydrolysis of various fats was observed in a number of in vitro studies. Meursing (1962) observed that the products of hydrolysis of natural fats and oils were more saturated than the fats from which the fatty acids were derived. Meinertz (1962, 1963) reported that, in the fatty acid mixture that is released from epididymal pads incubated with adrenaline, there are found lower proportions of oleic acid than in the fats from which the fatty acids are derived. Simultaneously, an accumulation of oleic acid occurred in the adipose tissue diglycerides. Meinertz (1962) observed that the magnitude of the differential release of the saturated and unsaturated acids was the same regardless of their position. This author suggested that the differential release of fatty acids is not due to the positional localisation, but to the specificity of fatty acids in the mechanism of release.

It is not known at present what is the precise role of

lipolytic enzymes in determining plasma FFA composition. Little is known about the intracellular pool of adipose tissue FFA (Jeanrenaud 1965). Hollenberg and Douglas (1962) have reported that the intracellular pool of rat adipose tissue contains more palmitic and palmitoleic, and less oleic and linoleic acids, than adipose tissue triglycerides. Miller et al., (1962) have made different observations with regard to dog adipose tissue; these authors have found the fatty acid composition of the total fraction to be similar to that of the FFA fraction.

In addition to the hypothesis discussed above on the influence of lipolytic enzymes on the composition of plasma FFA another theory has been advanced to explain the differences in the composition of adipose tissue fatty acids and plasma FFA. Miller et al., (1962) and Goldrick and Hirsh (1964) suggested that the differences are due to the fact that the fatty acids are modified in composition during their passage through the cell membrane. The work of Hollenberg and Angel (1963), however, indicates strongly that the transport of fatty acids through the cell membrane shows a preference for polar fatty acids, thus leading to increased proportions of unsaturated acids. These authors reported that when adipose tissue was incubated with corticotropin in medium containing albumin short-chain fatty acids were released faster than

long-chain, and that fatty acids with high degrees of unsaturation were released in preference to those with low degrees of saturation or to saturated fatty acids. According to these authors' opinion, the differences between the acids in their solubility in the medium provides the most obvious explanation for their results.

The mobility of individual fatty acids depends upon their affinity for albumin. Goodman (1958) has demonstrated that the affinity of fatty acids for albumin is influenced by the chain-length and by the degree of unsaturation. He studied the binding of a number of fatty acids in a partition system made up of an aqueous solution of albumin and n-heptane and observed three classes of binding sites. The first consisted of two sites, the second of five sites and the third of a number of sites arbitrarily assumed to be twenty. The association constants of fatty acids were in decreasing order of magnitude for oleic, stearic, palmitic and linoleic acid respectively.

It has been demonstrated in in vitro studies that the presence of albumin in the incubation medium is essential for fatty acid release from adipose tissue to occur. Even in the presence of the most powerful lipolytic hormones, no release of fatty acids could be obtained in the absence of albumin (Raben and Hollenberg, 1959).

(c) Efflux of FFA from the blood stream

Studies which employ isotope dilution techniques have shown that plasma FFA enter and leave the circulation in as short a time as 1 - 2 minutes (Fredrickson and Gordon, 1958a). Robinson (1964) summarised the evidence that, in the fasting state, about 40% of the fatty acids mobilised from adipose tissue are taken up by the liver. The remainder are taken by the peripheral tissues and directly oxidised. Originally, in their studies on humans, Fredrickson and Gordon (1958b) did not notice any differences between the rates of disappearance of palmitic, oleic and linoleic acids. Later, Usava et al., (1964) reported preferential removal of oleic acid. Studies of Göransson and Olivecrona (1964, 1965) and Göransson (1965 a, b, c) have established that, in the rat, the rate of disappearance of fatty acids from the serum increases with chain-length and saturation, and that the unsaturated 18 carbon fatty acids are extracted more readily than stearic or palmitic acids. Göransson (1965c) attributed this difference in the rates of disappearance to the more hydrophilic properties of unsaturated acids compared with saturated acids. According to these authors, the fact that fatty acid molecules have to pass through a water phase when transferred from the proteins in plasma to the surface of the cell causes the solubilities of individual fatty acids to determine their activity in the transportation system. So far, it is not known whether the

fatty acids pass the cell membrane protein-bound to albumin or as free fatty acids (Robinson, 1964). Göransson (1965c) suggested that the greater hydrophilic property of oleic acid, as compared with stearic or palmitic acid, might determine its more rapid disappearance from the blood.

(d) Extraction of FFA by different tissues

The rate of uptake of FFA by the liver is a function of plasma FFA concentrations (Stein and Shapiro, 1959; Kabal and Ramey, 1965). No differential uptake of fatty acids by the liver has yet been reported. Miller et al., (1962) did not observe any differences in the rate of uptake of stearic and oleic acids by dog liver. Differential uptake of fatty acids does, however, occur in the myocardium. Rothlin and Bing (1961), from in vivo studies, observed that, in the postabsorptive state, dog and human myocardium extracted higher percentages of oleic acid than any other fatty acid present in the arterial blood. This observation has been confirmed by in vitro studies (Evans et al., 1963; Evans 1964a, 1964b). The extent of myocardial extraction of individual fatty acids was inversely related to chain-length, and the monoenoic acids were extracted in preference to the saturated and dienoic acids. Evans (1964b) stated that the human heart, under conditions of hypoxia, did not show the preferential uptake of oleic acid that is usually present; all

acids were extracted at an equally slow rate.

Among other body tissues, electrically stimulated skeletal muscle has also been reported as taking up oleic acid in preference to other acids (Miller et al., 1962).

Concluding remarks

A number of metabolic functions other than those connected with the mechanism of the rate of influx and efflux may influence plasma FFA composition (incorporation into the various lipid classes, interconversion to other acids, synthetic and oxidation reactions etc.). It appears, however, reasonable to assume that, in the very first instance of fatty acid mobilisation, the changes in rate of transportation may play a predominant role in determining the pattern of serum FFA. Similarly, increased thyroid activity may stimulate, and decreased thyroid activity may inhibit, the rates of transportation of fatty acids between adipose tissue and the liver.

The following hypothesis is advanced as being the most likely explanation. Differences in the physical properties of fatty acids are of first importance in determining changes in plasma FFA composition. As proposed by Hollenberg and Angel (1963) and by Göransson (1965c), the fact that the fatty acid molecule has to pass from an aqueous compartment of the cell into plasma when released from adipose tissue, and in the opposite direction when extracted from plasma, may cause

the hydrophilic properties of fatty acids to decide on the relative rates of transportation. In this way, both oleic and linoleic acids should be transported in preference to saturated acids, and palmitic acid in preference to stearic acid. When the influx of FFA exceeds the efflux, it is likely that accumulation of acids preferentially released will result in increased percentages of these acids within the FFA fraction. In fact, the observations made immediately after noradrenaline infusion have shown parallel increases in FFA oleate and linoleate, and decreases in FFA stearate. These changes would be expected from the increased rate of FFA influx. The absence of parallel increases in FFA linoleic acid percentages in other conditions followed or accompanied by increases in serum FFA can be explained by the high metabolic activity of linoleic acid compared to oleic acid (incorporation into phospholipids, metabolism to arachidonic acid) or alternatively by its lower affinity for albumin compared with oleic acid.

STUDIES ON TRIGLYCERIDE FATTY ACIDS

Similarities and differences in the effect of various factors inducing fatty acid mobilisation

Changes in the percentage of triglyceride fatty acids (except those induced by antithyroid therapy in thyrotoxic patients) were much smaller than the corresponding changes in serum FFA. The results obtained from various studies are somewhat

conflicting. Higher degrees of monounsaturation of serum triglyceride fatty acids were observed in thyrotoxicosis and after acute myocardial infarction. Higher degrees of saturation were observed after a daily dose of HGH and in acromegaly. One could assume that thyroid hormones promoted the increases of triglyceride oleate, while HGH did not. However, the results of the effects of thyroid hormones are not completely clear.

Antithyroid therapy induced a significant lowering of triglyceride oleate associated with decreases in serum FFA concentrations, but no statistically significant increases in triglyceride oleate were found when hypothyroid subjects were treated with thyroid hormones. It appears that some persons may not be capable of raising the triglyceride oleate in response to increases in serum FFA to the same extent as others. This inability to raise triglyceride oleate in parallel with increases in serum FFA has been observed in patients suffering from chronic ischaemic heart disease.

To my knowledge, the effect of hormones on the composition of serum triglyceride fatty acids has not yet been described. The effects of hormones were studied in relation to adipose tissue triglycerides, and these will be discussed in the next paragraph.

In ischaemic heart disease, the present results agree with those reported by Bottcher and Woodford (1961) and do not show any significant differences in the pattern of the triglyceride fatty acids from the healthy controls, but they do show differences in the relative pattern of serum FFA and triglyceride fatty acids. No increases corresponding to those in free oleic acid percentages are found in triglyceride oleate, hence the smaller difference between the percentage levels of triglyceride and FFA oleic acid in the diseased group. Bottcher and Woodford (1961) observed somewhat less oleic acid in the triglycerides in the combined I-III Cohn fractions and somewhat more oleic acid in the FFA in the combined IV-V Cohn fractions in diseased subjects. Thus, their results agree with those presented here in indicating a smaller difference between the percentage levels of triglyceride and FFA oleic acid in the diseased subjects.

Interpretation of results

(a) Influence of serum FFA on serum triglyceride levels

Evidence from numerous isotopic studies has demonstrated that, in the fasting state, serum triglycerides derive mainly from the liver (Laurell, 1960; Havel and Goldfien, 1961; Robinson, 1963), where they are synthesised from plasma FFA and glycerophosphate, and released again into the plasma as part of the circulating low density lipoproteins (Stein and Shapiro, 1960; Havel and Goldfien, 1961; Borgstrom and

Olivecrona, 1961). After the injection of animals with labelled acetate (Harper et al., 1953) or labelled fatty acids (Havel and Goldfien, 1961; Borgstrom and Olivecrona, 1961; Orth et al., 1961) radioactivity appears in the plasma triglycerides. The maximum radioactivity after the injection of labelled fatty acids was recovered in plasma triglycerides after 40 minutes in rats (Laurell, 1959), after 30 to 60 minutes in dogs (Orth et al., 1961), and after 2 hours in man (Nestel, 1965).

The stimulation of mobilisation of serum FFA from adipose tissue by hormones has been shown to influence the concentrations of hepatic and serum triglycerides. Large increases in the hepatic triglyceride content were observed one hour after the administration of noradrenaline to rats, (Dury and Treadwell, 1958) and within 6 hours after the administration of growth hormone (Greenbaum and McLean, 1953). Elevation of plasma triglycerides after infusion of noradrenaline was reported in dogs (Feigelson et al., 1961) and in human subjects (Dury and Treadwell, 1958). More recently, Grasso et al., (1963) observed that these increases were higher in hyperlipidaemic subjects and in subjects with biliary cirrhosis (Grasso et al., 1964). Nestel (1964) gave evidence for the concept that the levels of plasma triglycerides in any individual are related to the responsiveness of the adipose tissue to catecholamines. A relationship was found between the fasting

plasma triglyceride concentrations and the increments in serum FFA after a 15-minute infusion of noradrenaline.

(b) Differentiation in the turnover rate of plasma FFA to plasma triglycerides

Little is known about the metabolic differences in conversion rates of individual plasma FFA to plasma triglyceride fatty acids. Orth et al., (1961) reported that both linoleic - C^{14} and palmitic - C^{14} acids, injected to dogs, were incorporated into plasma triglycerides at comparable rates and continued at similar concentrations for 72 hours. Nestel (1965), also using isotopic studies, reported different fractional turnover rates for palmitic and linoleic acids. That of linoleic only was inversely related to triglyceride concentrations. He concluded that some forms of hyperlipidaemia were accompanied by a corresponding increase in the turnover rate of palmitate, but not of linoleate. Whyte et al., (1963) showed that, during fat absorption in rats, triglyceride incorporation had a marked preference for stearic and linoleic acids compared with palmitic acid, and a relative discrimination against oleic acid. The authors showed that the α and β -positions of triglyceride were nearly identical with regard to fatty acid distribution of mass and radioactivity (which is not the case with lecithins).

(c) Efflux of triglycerides from the plasma

It is generally accepted that the fatty acids of plasma triglycerides represent the form in which fatty acids are transported from the liver to adipose tissues and other body sites (Carlson and Ekelund, 1963). In the rat, it has been shown that injected labelled triglycerides have a rapid turnover rate with an initial half-life of 3 - 5 minutes (Laurell, 1959). Recent studies have suggested that the disappearance rate of plasma triglycerides varies between subjects and depends upon the size of triglyceride lipoproteins (Quarfordt and Goodman, 1966). At normal plasma triglyceride concentrations the rate of removal has been calculated to be about 2 g per hour (Reaven et al., 1965). The isotopic studies revealed that much of the low density triglyceride lipoproteins circulated back and forth between the plasma and the liver (Carlson and Ekelund, 1963).

(d) Hormones and adipose tissue fatty acids

It has already been stated that adipose tissue triglycerides derive partly from synthesis within adipose tissue, and partly from synthesis in the liver, since they are transported in the blood stream from the liver to adipose tissue. A change in the composition of adipose tissue fatty acids might, therefore, be expected under the influence of agents promoting a higher mobilisation of serum FFA. This suggestion has been confirmed by studies on adipose tissue fatty acids. Rabinowitz

et al., (1965) reported small increases of adipose tissue oleic acid after treatment of obese men with thyroid hormones. Ellefson and Mason (1964) found that rat adipose tissue contained a higher percentage of oleic acid in the hyperthyroid state and a lower percentage in the hypothyroid state; the decreases in hypothyroid animals were particularly marked. All these observations demonstrate that thyroid hormone controls the proportion of adipose tissue oleic acid along the same lines as it controls the proportions of serum free and triglyceride fatty acids, as shown in the present investigation. Thus oleic acid, compared to saturated acids, is more rapidly transported, and one could speculate that such a trend in the FFA and triglyceride fatty acids might represent a more rapid rate of recycling of fatty acids between adipose tissue and liver.

As far as adipose tissue oleic acid is concerned, it is of particular interest that Krut and Bronte-Stewart (1963) reported that females had higher percentages of oleic and palmitoleic acids in their adipose tissue than males. If the suggestion that adipose tissue oleic acid represents a measure of the rate of fatty acid recycling is true, it would be expected that in females there should be a greater response to the influence of fatty acid mobilisation than in males. No such observation has so far been reported. Evidence provided by one study, however, indicates such a possibility: Quabbe et al., (1966) found that in females, during a 24-hour fast, both serum

FFA and serum HGH were at peak levels more frequently than in males.

Suggestion of a more probable explanation for the different effects of thyroid hormone and of HGH on triglyceride fatty acids

Higher triglyceride oleate was observed in the present work as a result of an excess of thyroid hormone and as a result of acute myocardial infarction, while higher triglyceride saturation (palmitate and stearate) was observed as a result of daily doses of HGH and in acromegaly. One speculative explanation for these observations is provided by the different effects of thyroid hormones and of HGH upon carbohydrate metabolism. For example, an excess of thyroid hormones leads to increased glucose utilisation and increased insulin degradation (Danowski et al., 1964). Different trends were described after daily administration of HGH. Wallace and Basset (1966) have recently observed that the daily administration of HGH causes rises in blood glucose and insulin. Patients with acromegaly are reported to be more resistant to insulin than normals in respect of plasma glucose levels (Landon et al., 1966). Thus, it is possible that higher concentrations of glucose and insulin in plasma will promote the saturation of triglyceride fatty acids, while hypermetabolic conditions will promote a relatively lower degree of saturation of triglyceride fatty acids.

The different influences on the rates of transportation, as already discussed, may additionally influence the pattern of

triglyceride fatty acids in different states of thyroid activity.

STUDIES ON PHOSPHOLIPID FATTY ACIDS

Similarities and differences in the effects of various factors inducing fatty acid mobilisation

No common pattern of change in the fatty acid composition of serum phospholipids can be recorded for those influences studied. It is of interest that under some conditions, accompanied by increases in serum FFA and by increased serum FFA oleic acid percentages, the percentages of phospholipid oleate were decreased. This was observed in hypothyroid patients following the treatment with thyroid hormones; daily injections of HGH produced a similar effect.

Changes in the FFA concentrations appear to be associated mostly with changes in phospholipid palmitate and phospholipid arachidonate in certain conditions. Increases in phospholipid palmitate were observed when an overnight fast was prolonged by 8 hours. High phospholipid palmitate percentages were recorded in patients with chronic ischaemic heart disease and with hyperlipidaemia, as well as immediately after an acute myocardial infarction. The similarity of trends in the two conditions, accompanied by different concentrations of serum phospholipids, indicates that the levels of plasma FFA may influence the described change in composition more than serum

phospholipid concentration.

The percentage of phospholipid arachidonate appears to be associated with the activity of thyroid hormones, as well as with the activity of noradrenaline. Since changes in the percentage of phospholipid arachidonate were accompanied, in most conditions, by changes in the percentage of cholesteryl arachidonate, they will be discussed together in the paragraph entitled "Studies on cholesteryl fatty acids".

I could not find similar studies on the effect of hormones or of fasting on the composition of serum phospholipid fatty acids, nor have changes immediately after myocardial infarction been reported. However, the pattern of phospholipid fatty acids in chronic ischaemic heart disease and in hyperlipidaemia have been reported (Schrade et al., 1961) and the significant increases in phospholipid palmitate and decreases in phospholipid linoleate observed here confirm these earlier findings.

Interpretation of results

Factors influencing the composition of serum phospholipids

There is little evidence concerning the various factors which determine the composition of serum phospholipids. Ansell and Hawthorne (1964) have recently summarised evidence for

the concept that plasma lipoprotein phospholipids are formed in the liver, removed from plasma by the liver and destroyed in the liver. In the liver the phospholipid lecithins are synthesised via the choline-cytidine pathway or via the methylation pathway and, in the latter, sex differences were observed (Bjørnstad and Bremer, 1966). The turnover of hepatic phospholipids was reported to be under hormonal influences. Thyroid hormone was reported to increase the turnover of phospholipids in the liver of adult rats (Flock et al., 1948), and noradrenaline in the rabbit liver (Dury, 1955).

Little is known about the specificity of enzymes catalysing the esterification of hepatic lecithins. Dustin et al., (1961) have shown preferential incorporation of linoleic rather than palmitic acid into hepatic lecithins when dogs were injected simultaneously with 1-C^{14}-linoleic and with 9, 10-H^{3}-palmitic acid. Nestel and Steinberg (1963) have shown that rat liver perfused with high concentrations of palmitic and linoleic acids incorporates palmitic acid at a greater rate into hepatic triglycerides, and linoleic acid into hepatic phospholipids.

Specificity for esterification of phospholipids was also reported for intestinal phospholipids, during fat absorption: Whyte et al., (1963) showed that esterification was highest for stearic and linoleic acids, lower for palmitic acid, and lowest for oleic acid.

The composition of plasma phospholipids can also be influenced by transesterification reactions between the lecithins and free cholesterol which takes place in the plasma. Glomset and his associates (1962, 1963) have reported the existence of a lecithin:cholesterol acyltransferase in plasma which stimulates the transfer of fatty acids from the β -position of lecithin to the cholesterol molecule. Since the β -position of lecithin is mostly occupied by the unsaturated acids, such as linoleic and arachidonic, the presence of this enzyme in plasma will result in a decrease in phospholipid unsaturated acids and in an increase in cholesteryl unsaturated acids.

Concluding remarks

The explanation of the changes in phospholipid fatty acids is more difficult than for the other fractions of serum lipids. It is not known whether the changes observed during the present studies were due solely to changes in the fatty acid composition of lecithins, or to changes in the relative proportions of lecithins, sphingomyelins, phosphatidylethanolamine or lysolecithins. Even assuming that they result from changes within the lecithin fraction only, evaluation of the mechanism is difficult. The different pathways of hepatic synthesis, turnover rates of hepatic phospholipids, rates of efflux from plasma, specificity for esterification and many other factors may account for the percentages in the fatty acid moiety in serum lecithins.

It is, however, noteworthy that it has been recently reported that the ratio of lecithin palmitate to stearate accounts for one of the sex characteristics in the rat: male rats had a higher ratio of palmitate to stearate than females (Aftergood and Alfin-Slater, 1967). The same characteristic increases in palmitic/stearic ratios were observed in the present study in the hypothyroid group and in the group of subjects with ischaemic heart disease, both chronic and acute. Thus, the hypothyroid state in the rat was accompanied by a decreased turnover of hepatic phospholipids (Flock et al., 1948). By comparison, one could speculate that the high ratio of phospholipid palmitate to stearate reflects the lower turnover rate of hepatic phospholipids. Alternatively, one can accept the explanation offered by Aftergood and Alfin-Slater (1967) for the similar characteristics in the rat. According to these authors, the differences observed in male and female rats reflect the different pathways of hepatic synthesis.

STUDIES ON CHOLESTERYL FATTY ACIDS

Similarities and differences in the effect of various factors inducing fatty acid mobilisation

Increases in serum FFA levels due to thyroid activity, or due to an induced mobilisation of serum FFA on the previous day either by HGH or myocardial infarction, have in most cases led to increases in cholesteryl oleate and decreases in cholesteryl linoleate. Patients with higher than normal levels of serum FFA,

such as those with chronic ischaemic heart disease,also have an increased percentage of cholesteryl oleate and a decreased percentage of cholesteryl linoleate. The opposite changes occurred as a result of antithyroid therapy in all thyrotoxic subjects, irrespective of their diet (when the serum FFA were significantly reduced). An increase in cholesteryl oleate was often associated with a rise in cholesteryl palmitate.

Increases in cholesteryl saturated and monounsaturated acids and decreases in di- and tetraunsaturated acids generally occur in conditions associated with hypercholesterolaemia (Goodman, 1965) and can be induced by dietetic experiments (see Introduction). These characteristics, if exaggerated, lead to an essential fatty acid deficiency. Not all stimuli of fatty acid mobilisation studied here act in this manner. Thyroid hormone activity does not lead to an essential fatty acid deficiency, since decreases in the percentage of linoleic acid are compensated by increases in the percentage of arachidonic acid, another essential fatty acid. High levels of cholesteryl arachidonate characterised the hyperthyroid state, and could be significantly reduced by antithyroid therapy. Low levels of cholesteryl arachidonate characterised the hypothyroid state, and could be significantly increased by treatment with thyroid hormones. These changes occurred irrespective of the diet of the patients under study.

Changes producing higher percentages of cholesteryl or phospholipid arachidonate, or both, have been observed in the present work in other conditions, such as during the infusion of noradrenaline, occasionally after prolonged fasting, and after myocardial infarction.

I could find only one report dealing with the effects of hormones on the composition of cholesteryl fatty acids. Boyd (1963) reported that thyroid hormone therapy in men increased the percentage of cholesteryl palmitate, palmitoleate and arachidonate, but not of oleate. With respect to the increases in cholesteryl arachidonate the present results are in agreement with those reported by Boyd.

Interpretation of results

Factors influencing the composition of serum cholesteryl fatty acids

The turnover of plasma FFA into cholesterol ester fatty acids measured by isotopic studies is slower than into triglyceride or phospholipid. After the injection of labelled palmitate or linoleate into dogs, the peak of radioactivity appears after 30 - 60 minutes in plasma triglycerides, after 4 - 8 hours in plasma phospholipids and after 25 - 36 hours in plasma cholesteryl fatty acids (Orth et al., 1961).

Factors which govern the composition of serum cholesteryl fatty acids are largely unknown. Some of these factors were recently studied in rat and man, and these studies are helpful in

the understanding of the present observations.

Plasma cholesterol esters derive from two main sources - the liver and the plasma. The liver serves as a chief source of plasma cholesterol ester synthesis (Friedman and Byers, 1955, Goodman and Shiratori, 1964). The evidence for this concept has been recently summarised by Nestel and Couzens (1966).

The cholesterol esters synthesised in the plasma by the plasma enzyme acyltransferase (Glomset et al., 1962, Glomset, 1963) from free cholesterol and the fatty acids occupying the β -position of lecithins account for an unknown portion of plasma cholesterol esters (Nestel and Couzens, 1966).

Differentiation in the esterification of cholesterol esters

In the rat liver, enzymes responsible for cholesterol ester synthesis have been shown to display a specific activity for the formation of cholesteryl oleate. Cholesteryl oleate was predominantly synthesised when rat liver was perfused in a medium containing albumin and an equimolar mixture of different fatty acids (Goodman et al., 1964). The esterification was in the order: cholesteryl oleate, palmitate, stearate and linoleate. This property of forming esters predominantly with oleic acid has also been demonstrated in in vivo experiments with rats. Oleate was the predominant cholesterol ester which accumulated in the rat liver irrespective of the fatty acid composition of the diet (Swell et al., 1964). When rats were injected with mevalonate-2-C^{14}, the rate of

appearance of the monounsaturated esters was greater than of any other esters (Goodman and Shiratori, 1964).

In the rat, this specificity in relation to the formation of cholesteryl monounsaturated acids was associated with the specific activity of turnover of these esters in plasma and liver (Goodman et al., 1964). The turnover of cholesterol esters in rat plasma is determined also by the lipoprotein fraction and, in the fraction of density d > 1.063, all cholesterol esters have a slower turnover (Gidez et al., 1967). In man, plasma cholesterol ester turnover rates are not determined either by lipoprotein class or by the fatty acid moiety (Goodman, 1964; Nestel and Couzens, 1966).

Formation of cholesteryl and phospholipid arachidonate

There is evidence in the literature supporting the view that the formation of plasma cholesteryl arachidonate is connected with hormonal activity.

Ellefson and Mason (1964) reported large increases in hepatic concentrations of cholesteryl and phospholipid arachidonate and of plasma arachidonate in hyperthyroid rats, and thought they were due to increased metabolism of linoleate to arachidonate under the influence of increased thyroid activity

Other factors were also reported to influence the levels of plasma arachidonate. Increases in cholesteryl arachidonate were observed in fasted rats (Boyd, 1963; Alberts and Gordon, 1962). A reduction in cholesteryl arachidonate was reported in

ovariectomised female rats (Boyd, 1963). Increases in cholesteryl arachidonate have recently been reported after treatment of depressed patients by electroconvulsive therapy (Cochran et al., 1965). Recently, Aftergood and Alfin-Slater (1965) have reported that, in the rat, conditions associated with low levels of plasma cholesterol were generally accompanied by higher total plasma arachidonate concentrations.

Some authors have observed sex differences in the formation of arachidonic acid. Lyman et al., (1964) reported that female rats, and castrated male rats treated with oestrogen, maintained higher proportions of arachidonate in the plasma during essential fatty acid deficiency than intact males or testosterone-treated castrates. More recently, Aftergood and Alfin-Slater (1967) reported that stimulation of the conversion of linoleic to arachidonic acid during the incubation of rat plasma occurred only in plasma from female rats. These authors concluded from their results that the formation of arachidonic acid was related to the presence of acyltransferase in rat plasma, and that the transesterification process might be depressed by androgen or enhanced by oestrogen.

Concluding remarks

The observed increases in the percentage of cholesteryl oleate in association with high levels of serum FFA, or following rapid increases in serum FFA, may be related to the fact that

oleic acid is in these conditions supplied to the liver in higher proportions by the circulating fluids. If cholesteryl oleate is formed in greater amounts than other esters, after increases in serum FFA, and if it cannot be hydrolysed more rapidly than other esters (as shown in the rat), the conditions accompanied by repetitive increases in serum FFA may promote mainly the formation of cholesteryl oleate.

The present study has shown that levels of cholesteryl and phospholipid arachidonate are materially influenced by, and controlled by, thyroid hormones. The effects of thyroid hormones on the formation of arachidonic acid may be mediated through endogenous noradrenaline, which leads to a similar response when infused. It appears that the mechanism involved in the formation of plasma arachidonate is connected in some way with factors which influence the susceptibility of adipose tissue to catecholamine-stimulated FFA mobilisation.

THE CONTRIBUTION OF THE PRESENT WORK TO THE PROBLEM OF ATHEROSCLEROSIS

Increased fatty acid mobilisation may be involved in the development of atherosclerotic vascular disease by various mechanisms. The possible explanation presented by Carlson et al., (1965) illustrates schematically three main sites of action for increased fatty acid mobilisation.

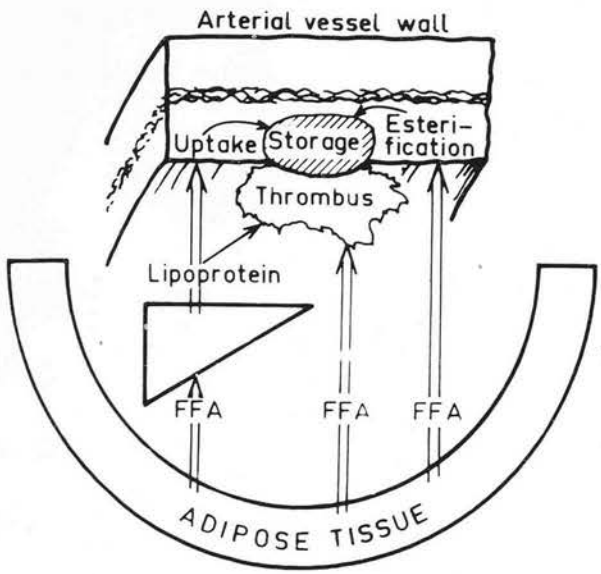


FIG. 9. Possible sites of action for increased lipid mobilization in the development of atherosclerosis. Schematically indicated is the possible effect of increased lipid mobilization on the lipid content of the intima, on thrombogenesis and on the hepatic formation of lipoproteins.

From Carlson, Boberg and Hogstedt (1965)

It is not known at present whether the hyperlipoproteinaemia seen in ischaemic heart disease promotes the development of the disease or is only a symptom (Carlson et al., 1965). A few studies will be cited which support the concept that plasma cholesterol esters may contribute to the development of atherosclerosis.

Experiments with rabbits fed cholesterol have shown that the increases in serum cholesteryl oleate which resulted were

associated with the accumulation of cholesteryl oleate in aortic atheromatous plaques (Zilversmit et al., 1961). In the human, advanced atherosclerosis has been shown to be associated with relatively high proportions of cholesteryl linoleate in the walls and plaques (Böttcher et al., 1960; Mead and Gouze, 1961; Böttcher and Woodford, 1962). Evidence from other study on human aortic lipids has shown that areas of fatty streaks contained relatively more cholesteryl oleate than normal intima and media from the same aorta (Nelson et al., 1961). Also, it has been shown that the rate of exchange between arterial cholesterol esters and between plasma cholesterol esters is slower in atherosclerotic segments (Chobanian and Hollander, 1964). Thus, the studies of Zilversmit et al., (1961) and Nelson et al., (1961) indicate that cholesteryl oleate may contribute more than other cholesterol esters to the development of atherosclerosis.

In several pathological conditions accompanied by an accumulation of cholesterol esters in tissues, marked accumulation of cholesteryl oleate has been reported. It predominated in the cholesterol esters in a bone marrow lesion in Hand-Schuller-Christian disease (Fredrickson et al., 1961), in tissues from patients with Tangier disease (an α-lipoprotein deficiency ; Fredrickson et al., 1961) and in human xanthomata (Fletcher and Gloster, 1964). Thus, these studies indicate that cholesteryl oleate has a greater tendency to accumulate in various tissues than any other cholesterol ester.

The results of the present study aid the understanding of atherosclerosis and ischaemic heart disease by the following observations:

1. It has demonstrated that patients with ischaemic heart disease have on average increased percentages of oleic acid in their serum FFA. This characteristic has not previously been described. This is also true when FFA are mobilised by such stimuli as thyroid hormones, fasting, HGH injections (associated with fasting), acute myocardial infarction or noradrenaline.

It is interesting that the composition of serum FFA in patients after acute myocardial infarction and to lesser extent in patients with chronic ischaemic heart disease is similar to that induced by noradrenaline infusion or that observed in increased thyroid activity. Patients with chronic ischaemic heart disease have been reported as having a greater than normal secretion of noradrenaline (Arlow, 1945; Friedman et al., 1960; Starich and Abbanelli, 1959; Gazes et al., 1959). It has been suggested that they react to stressful situations more frequently (Carlson et al., 1965) and with a greater mobilisation of plasma FFA; they had higher levels of plasma FFA after cigarette smoking (Kershbaum et al., 1961) and in response to the minor stress of testing their capacity to perform feats of mental arithmetic (Pecnik, 1962). The similarity of effects induced during a noradrenaline infusion with those which occur after acute myocardial infarction and in some patients

suffering from chronic ischaemic heart disease supports the concept that increased levels of plasma noradrenaline may be responsible for the changes in these patients.

2. The absence of increases in triglyceride oleate corresponding to increases in free oleic acid in patients with chronic ischaemic heart disease permits one to speculate that in these patients, unlike normal healthy controls, the fatty acids carried back to adipose tissue, compared with the fatty acids removed from adipose tissue, are more saturated and less monounsaturated. This may be due in part either to disturbed carbohydrate metabolism, or to the slower rate of fatty acid transportation, as was discussed in relation to the hypothyroid state.

3. Patients with chronic ischaemic heart disease have higher percentages of oleic and lower percentages of linoleic acid in serum cholesterol esters. These characteristics have been described earlier in association with hypercholesterolaemia, but not in association with conditions accompanied by or followed by, a higher mobilisation of serum FFA. As has already been stated, the increased availability of oleic acid in the circulation, the increased specificity for formation of cholesteryl oleate, and lack of specificity for the turnover of cholesteryl oleate may create favourable conditions for the increased percentages of cholesteryl oleate. It is noteworthy that species that are less susceptible to atherosclerosis, such as the rat and dog, have less cholesteryl oleate in their serum cholesterol ester

fractions than has man.

4. The fatty acid composition of serum lipids has been measured soon after myocardial infarction and on two consecutive subsequent days. The changes, not previously reported, which were observed here in the free and triglyceride fatty acids were similar in character to those of hyperthyroid patients. As in hyperthyroid patients, high values for free and triglyceride oleic acid were observed. In the cholesteryl fatty acids there were decreases in linoleic acid of the same magnitude as in hyperthyroid subjects; however, only in two out of the eight patients studied were there corresponding increases in cholesteryl arachidonate. The phospholipid fatty acid pattern resembled that of chronic hyperphospholipidaemia. On the later days of the illness, between the first and the second day increases in free and triglyceride oleate were observed. In the two patients most severely affected (one died on the third day and one was unconscious on the second day) there was a marked rise in saturated acids and a decrease in monounsaturated acids in the FFA fraction, unaccompanied by any significant changes in FFA linoleic acid which could obscure the higher degree of saturation observed. It is not known whether this different reaction in two of the eight subjects with myocardial infarction was associated with the severity of the illness. Much more evidence must be collected before any conclusions can be reached on the

sequence of changes in this disease.

5. The question arises why, in all patients with chronic ischaemic heart disease having high levels of serum FFA, and in all those after an acute myocardial infarction, there is not a rise in cholesteryl or phospholipid arachidonate. Rises in phospholipid or phospholipid and cholesteryl arachidonate were observed after noradrenaline infusion. Could there be a deficient formation of arachidonic acid in these patients, as has been observed in hypothyroid subjects?

It has been previously observed that there is a correlation between the levels of arachidonic acid in the serum cholesteryl esters and species susceptibility to atherosclerosis. Those species (rat and dog) with high levels of arachidonic acid in their serum cholesterol esters are known to be more resistant to the experimental production of atherosclerosis, while more susceptible species (man, chicken, goose, rabbit and guinea-pig) have low levels of this acid (Swell et al., 1960). Since hyperthyroid persons with high serum cholesteryl arachidonate are less susceptible to atherosclerosis than hypothyroid ones, the present observations support the theory developed by Swell et al., (1960).

As yet it is impossible to extrapolate from speculations to conclusions. This thesis has concentrated on plasma levels, and now these findings must be related to adipose tissue and hepatic levels. Future studies on adipose tissue fatty acids,

the intracellular pool of FFA, responsiveness to lipolytic factors, cell permeability for fatty acid transport, and rates of esterification are all likely to yield valuable information on the factors responsible for the observations in this study.

(i)

SUMMARY

A modified technique for separation of serum free fatty acids (FFA), triglycerides, phospholipids and cholesterol esters by adsorption chromatography on a system of two columns, silicic acid and Florisil, has been elaborated for preparing samples for gas-liquid chromatographic analysis of serum lipid fatty acids.

An attempt was made to study the changes in fatty acid composition of the various lipids in response to factors influencing fatty acid mobilisation. The following stimuli of fatty acid mobilisation were investigated: thyroid hormone, human growth hormone (HGH), noradrenaline, prolonged fasting, and acute and chronic ischaemic heart disease.

The effects of thyroid hormones were studied 1) after administration of LT_3 to normal subjects; 2) after thyroid hormone therapy to hypothyroid subjects and 3) after antithyroid therapy to thyrotoxic subjects.

The effects of HGH were studied 1) during the daily administration of HGH; 2) hourly after HGH injection; and 3) in patients with acromegaly.

The effects of noradrenaline were studied during its infusion.

The effects of fasting were studied when an overnight fast was prolonged by 8 hours.

(ii)

The effects of acute ischaemic heart disease were studied on the day of myocardial infarction and on two subsequent consecutive days. The effects of chronic ischaemic heart disease were studied in patients with angina pectoris, and those with a past history of myocardial infarction. One control group consisted of middle-aged males, and a second of post-menopausal females.

Percentage composition studies have revealed that increased mobilisation of serum FFA due to any of the adipokinetic factors studied was accompanied by statistically significant increases in the percentage composition of serum FFA oleic acid. These increases in oleic acid percentages were mostly accompanied by parallel decreases in the percentage of FFA saturated acids: stearic and palmitic acids. It is proposed that increases in the percentage of FFA oleic acid were due to its higher activity in transportation compared with saturated acids, in the aqueous medium of adipose cells and plasma. This is thought to be due to the physical properties of oleic acid, its greater hydrophilic property and also, possibly, its greater affinity for albumin.

The changes in triglyceride fatty acids were not uniform for all the influences studied. Triglyceride oleate was significantly lowered by antithyroid therapy in hyperthyroid subjects, but no

(iii)

significant increases could be proved as a result of thyroid therapy in hypothyroid subjects, thus suggesting that the increased incorporation of oleic acid into triglyceride fatty acids may be inhibited in some subjects. Administration of HGH did not affect triglyceride oleate; observations made during daily administration of HGH, and in the disease of acromegaly, suggest that HGH may promote a higher degree of saturation in triglyceride fatty acids. In subjects with acute myocardial infarction, the triglyceride fatty acid pattern resembled that of hyperthyroid subjects. In chronic ischaemic heart disease, the pattern of triglyceride fatty acids did not differ from normal. The lack of parallel increases in percentages of triglyceride oleate with increases in percentages of FFA oleic acid in patients with chronic ischaemic heart disease suggests that incorporation of oleic acid into triglyceride fatty acids in these patients is possibly lower.

In the phospholipid fatty acids, similarly, no common pattern of changes was noted for the different stimuli studied. The percentage of phospholipid palmitate decreased significantly immediately after noradrenaline infusion. Opposite changes were noted after prolonged fasting. Equally high levels were observed shortly after myocardial infarction as in ischaemic patients with hyperphospholipidaemia. The percentage of phospholipid arachidonate increased significantly following

(iv)

thyroid hormone therapy in hypothyroid subjects. Phospholipid arachidonate also showed an immediate rise during noradrenaline infusion. The latter observation supports the concept that thyroid hormone activity may be mediated through the action of endogenous catechclamines.

The percentage composition studies on cholesteryl fatty acids suggest that changes in serum FFA concentrations (due to HGH daily dose, thyroid hormone and antithyroid therapy, acute myocardial infarction) produce in most cases directly-related changes in cholesteryl oleate percentages and inversely-related changes in cholesteryl linoleate. It is probable that the increased mobilisation of serum FFA, accompanied by predominant increases in FFA oleic acid, may promote the formation of cholesteryl oleate. Cholesteryl arachidonate was shown to be materially influenced and controlled by thyroid function: a significant increase in the percentage of cholesteryl arachidonate occurred after thyroid hormone therapy in hypothyroid subjects, and a significant decrease occurred after antithyroid therapy in thyrotoxic subjects; the thyrotoxic patients had percentage levels of cholesteryl arachidonate twice as high as those of the hypothyroid patients. The results agree with the suggestion of Ellefson and Mason (1964) that thyroid hormones stimulate an increased synthesis of arachidonic acid from linoleic acid.

(v)

An attempt was made to discuss the results in relation to certain metabolic studies, and to postulate possible implications of the predominant increase in serum FFA oleic acid percentage at the time when fatty acids are mobilised from the storage sites.

APPENDIX

APPENDIX TABLE 1.

The effect of administration of LT_3 (3,5,3'-triiodo-L-thyronine) on the percentage composition of serum esterified fatty acids in 2 euthyroid subjects

(Expressed as percentage of total fatty acids in the fraction)

Subject	Fatty acid short hand design.	Free Fatty Acids							Triglyceride Fatty Acids						
		Pre-treatment period		On treatment days			Days after last dose		Pre-treatment period		On treatment days			Days after last dose	
				3	5	7	3	9			3	5	7	3	9
		526	522	620	628	684	670	641	n.e.	1.30	1.16	1.01	0.99	0.94	1.33
T.H. male age 23	16:0								23	26	22	23	22	26	25
	16:1								3	3	3	3	4	3	4
	18:0	n.e.		n.e.			n.e.		6	5	7	5	3	4	5
	18:1								50	48	49	48	54	53	52
	18:2								16	16	16	17	15	13	12
	18:3								T	T	T	T	T	T	T
	20:3								T	T	T	T	T	T	T
	20:4								2	2	2	3	2	1	1
		Pre-treatment period		On treatment days			Days after last dose		Pre-treatment period		On treatment days			Days after last dose	
				4	7		3	9			4	7		3	9
		592	519	444	293		398	n.e.	1.62	1.30	1.06	0.98		1.55	1.48
J.P. male age 30	16:0								27	28	28	28		29	24
	16:1								6	6	7	5		6	7
	18:0	n.e.		n.e.			n.e.		5	4	3	3		3	4
	18:1								47	47	48	50		47	48
	18:2								12	12	12	11		12	14
	18:3								1	1	1	2		1	1
	20:3								T	T	T	T		T	T
	20:4								1	1	1	1		1	1

Subject	Fatty acid short hand design.	Phospholipid Fatty Acids							Cholesteryl Fatty Acids						
		Pre-treatment period		On treatment days			Days after last dose		Pre-treatment period		On treatment days			Days after last dose	
				3	5	7	3	9			3	5	7	3	9
		232	238	240	190	222	278	248	195	216	206	178	176	158	170
T.H. male age 23	16:0	26	26	26	22	28	27	27	10	10	11	11	10	9	9
	16:1	T	T	T	T	1	1	1	3	3	3	3	3	3	3
	18:0	15	15	17	16	16	16	15	1	2	1	1	1	1	2
	18:1	13	15	14	14	14	14	17	22	22	23	23	24	23	22
	18:2	29	27	23	25	21	24	24	55	54	52	53	50	50	51
	18:3	T	T	T	T	T	T	T	T	T	T	T	T	1	1
	20:3	4	4	4	4	4	4	3	1	1	1	1	1	1	1
	20:4	13	13	16	19	15	14	13	7	7	8	8	10	11	10
		Pre-treatment period		On treatment days			Days after last dose		Pre-treatment period		On treatment days			Days after last dose	
				4	7		3	9			4	7		3	9
		n.e.	n.e.	n.e.	n.e.		n.e.	n.e.	n.e.	217	185	171		173	178
J.P. male age 30	16:0	29	31	30	32		29	25	10	11	11	10		11	10
	16:1	2	1	1	1		1	1	4	5	5	5		4	3
	18:0	16	17	16	15		16	14	2	2	2	1		1	1
	18:1	17	15	18	17		15	17	22	22	22	20		20	18
	18:2	22	22	20	19		21	25	50	49	49	51		48	58
	18:3	T	T	T	T		T	T	2	1	1	T		1	T
	20:3	3	3	3	4		4	3	1	1	1	1		3	1
	20:4	10	10	11	12		13	14	8	8	8	11		12	8

Abbreviations: n.e. - not estimated
T - trace amount

The first position in the columns represents the concentration of total lipids in the serum: Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq/l.

APPENDIX TABLE 2.

Effect of treatment with thyroid hormones on the percentage composition of serum lipid fatty acids in 9 hypothyroid subjects.

(aged 50-60)

(Expressed as percentage of total fatty acids in the fraction)

Subject	Fatty acid short hand design.	Free fatty acids				Triglyceride fatty acids				Phospholipid fatty acids				Cholesteryl fatty acids			
		Untreated	On treatment			Untreated	On treatment			Untreatad	On treatment			Untreated	On treatment		
				A	B			A	B			A	B			A	B
Q.E.		655	900	839		5.52		2.48	1.44	485		385	318	486	452	295	237
A : 4 wks. tr.	16:0	32	33	23	26	28	26	26	28	33	27	27	32	13	12	12	13
B : 2 mths. tr.	16:1	6	6	4	5	9	9	9	9	2	2	1	1	5	6	8	7
	18:0	13	13	13	13	5	6	3	4	17	18	19	17	1	1	1	1
	18:1	41	42	54	51	48	50	53	49	15	17	17	15	32	31	30	27
	18:2	8	7	7	6	9	9	7	9	20	23	15	17	39	41	37	40
	18:3 + 20:0	T	T	T	T	1	1	2	1	T	1	1	T	1	1	2	1
	20:3	-	-	-	-	T	T	T	T	3	3	6	4	T	T	1	1
	20:4	1	1	T	T	T	T	T	T	10	10	14	13	6	7	9	9
P.M.		815	910		839	0.94	0.78		1.08		439		334	445	439		290
B : 3 mths. tr.	16:0	33	30		28	29	29		25	38	39		33	12	11		10
	16:1	4	3		4	6	5		4	1	1		1	5	5		5
	18:0	19	18		15	3	5		2	16	15		15	1	1		T
	18:1	33	37		45	48	47		53	17	18		16	22	22		20
	18:2	9	9		8	12	13		12	20	19		23	55	55		58
	18:3 + 20:0	1	3		T	1	1		1	T	T		T	T	T		1
	20:3	-	-		-	-	-		-	1	1		2	T	T		T
	20:4	T	T		T	1	1		2	7	7		9	4	5		6
K.B.			n.e.	n.e.	n.e.		2.97	1.74	1.83		n.e.	n.e.	n.e.		432	279	243
A : 7 mths. tr.	16:0		36	34	30		25	32	26		33	35	35		14	12	12
B : 11 mths. tr.	16:1		5	3	3		5	6	7		1	2	2		4	6	4
	18:0		14	14	16		6	4	6		14	13	14		2	1	1
	18:1		34	41	45		45	44	46		16	15	15		26	24	24
	18:2		6	6	6		15	11	9		26	22	21		50	50	53
	18:3 + 20:0		1	1	T		3	1	T		2	2	2		T	T	T
	20:3		-	-	-		T	T	T		2	4	3		T	T	T
	20:4		1	1	1		3	T	4		6	7	8		4	6	4
A.J.		580	770	1216	1126	2.91	2.72	0.95	1.42	352	318	320	337	405	398	320	330
A : 1 mth. tr.	16:0	32	36	33	31	35	35	29	27	32	30	34	29	13	16	12	11
B : 3 mths. tr.	16:1	6	5	4	4	8	8	8	6	2	2	2	1	10	11	5	4
	18:0	13	15	14	13	5	5	4	3	19	18	16	21	T	1	1	1
	18:1	39	37	44	44	40	42	46	46	19	21	14	14	26	29	22	19
	18:2	9	7	6	6	7	8	9	17	15	16	20	24	45	40	52	53
	18:3 + 20:0	-	-	T	1	T	T	T	T	T	T	T	T	T	T	T	T
	20:3	-	-	-	-	-	-	-	-	2	3	3	2	T	T	T	T
	20:4	1	2	T	1	3	T	3	T	9	10	12	8	5	4	9	9
S.M.			1430	1538			1.82	0.94			270	251			330	320	
A : 14 days tr.	16:0		29	27			31	31			34	43			14	14	
	16:1		7	8			7	6			2	2			5	6	
	18:0		12	12			4	3			14	12			1	1	
	18:1		44	47			48	51			17	14			23	25	
	18:2		9	6			10	10			18	16			47	44	
	18:3 + 20:0		T	T			T	T			2	1			1	1	
	20:3		-	-			-	-			1	1			T	T	
	20:4		-	-			-	-			11	11			7	8	
H.T.			n.e.	n.e.	n.e.	1.98	2.63	1.95	1.19	n.e.	n.e.	n.e.	n.e.	249	323	182	195
A : 6 wks. tr.	16:0		30	29	27	25	25	26	26	27	29	28	35	9	9	12	11
B : 11 mths. tr.	16:1		4	4	5	4	4	5	10	1	1	T	1	2	3	4	4
	18:0		13	20	16	5	5	5	3	16	16	15	16	1	1	1	1
	18:1		42	38	45	55	55	52	53	18	17	18	15	27	21	27	21
	18:2		10	8	6	11	11	11	8	24	23	23	17	52	54	44	54
	18:3 + 20:0		T	T	T	-	-	-	-	1	1	1	T	1	T	T	T
	20:3		-	-	-	-	-	-	-	2	2	3	4	T	T	T	T
	20:4		T	T	T	T	T	T	T	10	10	12	12	7	8	10	10
E.B.			406	640	564	0.76	0.85	0.64	0.74	n.e.	n.e.	227	262	259	242	217	219
A : 6 wks. tr.	16:0		29	29	26	27	26	27	33	35	30	31	32	13		11	12
	16:1		3	8	6	7	7	7	7	1	2	2	1	3		4	5
	18:0		21	14	17	5	11	5	5	17	16	15	18	2		1	1
	18:1		37	39	41	50	52	47	43	14	15	12	15	25		23	23
	18:2		10	9	9	10	10	12	11	20	25	20	22	52		52	51
	18:3 + 20:0		-	-	-	1	1	1	2	T	T	T	1	1		1	1
	20:3		-	-	-	-	-	-	-	2	1	7	2	T		1	1
	20:4		T	T	T	T	T	T	T	10	11	12	10	5		6	6
R.J.		520	490	683	602	1.66	1.36	1.10	1.60	337	300	170	220	311	294	224	236
3 mths. tr.	16:0	32	28	26	24	27	26	30	27	32	35	38	37	10	9	12	13
A and B	16:1	5	5	4	4	8	6	6	7	1	1	1	1	4	3	6	5
(Controlled	18:0	15	13	11	14	3	3	2	2	10	11	16	13	23	21	26	21
metabolic	18:1	42	42	50	46	49	52	52	53	20	20	16	16	25	25	26	26
conditions)	18:2	10	12	9	12	13	14	10	11	27	25	20	22	56	58	52	51
	20:3	T	T	T	T	T	T	T	T	2	1	2	3	T	T	T	T
	20:4	T	T	T	T	T	T	T	T	8	6	8	7	4	4	4	4
L.J.		480	722	1118	870	3.74	4.26	2.53	2.53	303	430	168	164	356	360	163	164
10 wks.	16:0	31	32	28	32	31	29	31	29	35	33	32	33	13	14	14	14
A and B	16:1	7	7	5	5	8	8	7	7	3	2	2	1	8	7	8	6
(Controlled	18:0	14	11	14	11	5	4	3	2	15	17	17	19	T	T	T	1
metabolic	18:1	46	44	52	47	49	51	54	55	20	19	19	19	28	27	30	30
conditions)	18:2	4	5	5	4	7	8	5	5	19	18	13	14	46	46	39	39
	20:3	T	T	T	T	-	-	T	T	T	T	T	T	T	T	1	T
	20:4	T	T	T	T	T	T	T	T	7	8	12	11	5	5	8	9

Abbreviations: T - trace amount
n.e. - not estimated

The first position in the columns represents the concentration of total lipids in the serum: Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acid concentrations in µEq/l.

APPENDIX TABLE 3.

The effect of treatment with antithyroid agents on the percentage composition of serum lipid fatty acids in 4 thyrotoxic subjects (age 50 - 60).

(Expressed as percentage of total fatty acids in the fraction)

Subjects	Fatty acid short hand design.	Free fatty acids				Triglyceride fatty acids				Phospholipid fatty acids				Cholesteryl fatty acids			
		Untreated		On treatment		Untreated		On treatment		Untreated		On treatment		Untreated		On treatment	
				A	B			A	B			A	B			A	B
J.R. female		n.e.	918	860	705	2.22	2.24	2.65	2.58	n.e.	212	302	n.e.	142	168	310	214
A : 5 mths. tr.	16:0	26	25	31	30	31	29	34	30	33	33	32	31	14	13	12	11
B : 8 mths. tr.	16:1	4	4	4	4	6	6	8	7	1	1	2	2	6	6	9	7
	18:0	14	13	13	16	3	2	3	3	19	20	16	17	3	2	1	T
	18:1	50	51	44	42	51	54	45	46	13	13	16	15	27	27	23	22
	18:2	5	6	7	7	9	8	9	12	17	16	20	24	41	43	50	55
	20:3	-	-	-	-	-	-	-	-	6	6	4	3	1	1	T	T
	20:4	T	T	T	1	T	T	T	T	10	10	9	8	7	7	5	4
J.H. male		682	835	677	607	1.25	1.27	n.e.	1.90	225	202	n.e.	298	136	118	n.e.	228
A : 6 wks. tr.	16:0	25	24	30	33	23	24	26	28	31	31	30	31	11	11	9	9
B : 3 mths. tr.	16:1	4	4	2	3	5	5	5	6	2	1	2	2	6	6	5	6
	18:0	13	15	21	17	3	4	6	5	15	16	15	15	1	2	1	1
	18:1	49	48	36	37	56	57	49	50	16	17	16	17	26	27	25	24
	18:2	7	6	7	8	9	8	11	10	18	19	24	23	44	42	51	51
	20:3	-	-	-	-	-	-	-	-	4	3	2	2	1	1	1	1
	20:4	1	2	3	1	2	1	2	2	13	13	10	9	10	10	8	7
M.A. female		770	910	588	627	1.01	1.02	1.20	1.85	236	220	n.e.	370	140	178	202	249
A : 6 wks. tr.	16:0	24	25	29	28	26	24	26	25	25	27	33	31	12	12	12	9
B : 3 mths. tr.	16:1	5	4	5	5	6	7	7	6	1	2	2	1	6	6	6	5
	18:0	10	10	10	11	4	4	4	3	17	16	16	15	1	T	1	1
	18:1	47	48	45	44	48	50	50	44	17	17	16	13	21	23	24	19
	18:2	11	10	10	10	13	12	10	19	19	20	17	24	49	47	48	59
	20:3	-	-	-	-	-	-	-	-	5	5	4	4	1	2	1	1
	20:4	2	2	1	1	2	2	2	2	15	14	11	11	9	9	8	6
W.H. male		1105	955	670	670	1.39	1.09	2.74	2.05	127	138	238	217	152	148	265	260
A & B: 2 mths. tr.	16:0	28	28	29	29	28	28	30	30	36	36	34	34	11	12	13	11
(Controlled	16:1	5	5	5	3	5	5	7	7	1	1	1	1	4	4	5	4
metabolic	18:0	10	10	15	14	4	4	4	4	16	16	16	17	1	1	1	1
conditions)	18:1	49	49	43	45	53	52	52	52	15	16	16	17	28	29	29	27
	18:2	8	8	7	7	10	10	8	8	16	15	18	18	45	43	45	44
	20:3	T	T	T	T	T	T	T	T	4	5	3	3	1	T	T	1
	20:4	T	T	T	T	T	T	T	T	11	11	9	9	10	9	6	5

Abbreviations: n.e. - not estimated
T - trace amount

The first position in the columns represents the concentration of total lipids in the serum:
Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq/l.

APPENDIX TABLE 4.

Effect of treatment with antithyroid agents on the percentage composition of serum esterified fatty acids in 3 hyperthyroid women (age 20 - 30).

(Expressed as percentage of total fatty acids in the fraction).

Subjects	Fatty acid short hand design.	Free fatty acids		Triglyceride fatty acids		Phospholipid fatty acids		Cholesteryl fatty acids	
		Untreated	On treatment	Untreated	On treatment	Untreated	On treatment	Untreated	On treatment
		860	398	1.18	1.08	n.e.	n.e.	172	197
M.N.	16:0			27	27	30	25	11	7
	16:1			5	5	1	1	5	3
5 weeks on	18:0			7	5	17	15	1	1
treatment	18:1	n.e.	n.e.	49	44	15	14	22	19
	18:2			12	16	19	24	50	57
	20:3			-	-	3	2	T	T
	20:4			2	2	15	18	12	12
		930	n.e.	1.15	1.89	n.e.	n.e.	217	232
M.L.	16:0			24	24	29	24	12	11
	16:1			4	5	1	1	5	5
6 weeks on	18:0			3	4	18	16	1	1
treatment	18:1	n.e.		51	48	17	16	24	23
	18:2			14	15	18	24	46	48
	20:3			-	-	3	2	T	T
	20:4			3	3	14	16	13	12
		1305	705	n.e.	n.e.	n.e.	n.e.	136	217
F.J.	16:0					25	23	13	11
	16:1					1	1	4	3
7 weeks on	18:0					16	15	1	1
treatment	18:1	n.e.	n.e.			16	15	22	18
	18:2					26	30	49	58
	20:3					3	2	-	-
	20:4					13	13	11	10

Abbreviations: n.e. - not estimated
T - trace amount

The first position in the columns represents the concentration of total lipids in the serum:
Total cholesterol concentration in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq/l.

APPENDIX TABLE 5.

Long-term effects of daily administration of HGH on the percentage composition of serum lipid fatty acids.

(Measured 24 hours after each HGH injection).

Subject	fatty acid short hand design.	Free fatty acids								Triglyceride fatty acids								Phospholipid fatty acids								Cholesteryl fatty acids							
		Pre-treatment		Days on-treatment				After last injection days		Pre-treatment		Days on-treatment				After last injection days		Pre-treatment		Days on-treatment				After last injection days		Pre-treatment		Days on-treatment				After last injection days	
				1	2	3	4	3-5	6 and over			1	2	3	4	3-5	6 and over			1	2	3	4	3-5	6 and over			1	2	3	4	3-5	6 and over
		454	448	678	487	459	393	369	598	2.25	1.46	2.44		2.18		n.e.	2.34	n.e.	n.e.		n.e.			n.e.	n.e.	254	246	211	211	205	203	185	265
J.H.	16:0									31	31	34		41			31	36	32		28			32	31	14	13	26		24		27	13
male	16:1									12	11	8		6			12	2	2		1			1	2	8	9	10		10		9	11
age 58	18:0	n.e.		n.e.				n.e.		2	3	5		3			4	15	15		18			18	17	1	1	2		1		1	T
(IHD)	18:1									45	47	45		46			42	19	16		17			20	18	28	29	40		36		41	27
	18:2									9	9	8		4			11	16	18		18			15	17	42	41	20		27		20	41
	20:3									-	-	-		-			-	2	4		4			3	4	-	-	-		-		-	-
	20:4									T	T	-		T			T	10	14		14			12	12	7	7	3		2		2	7
		479	523	684	553	479	338	367	688	1.34	0.90	0.70		0.84		1.44	1.80	n.e.	n.e.		n.e.			n.e.		198		180		170		152	
G.B.	16:0									33	33	33		34		31		30	29		29			31		12		14		17		13	
male	16:1									8	8	8		6		7		2	2		2			2		6		7		6		7	
age 18	18:0	n.e.		n.e.				n.e.		6	6	6		7		5		16	14		15			15		1		2		3		3	
(healthy)	18:1									43	43	42		42		47		19	17		16			15		24		24		34		24	
	18:2									10	10	11		11		10		19	20		23			20		51		47		37		47	
	20:3									-	-	-		-		-		2	4		3			2		T		-		-		-	
	20:4									T	T	T		T		T		12	13		13			14		6		6		3		6	
		328	528	712	768	574	383	317	489	1.73	1.64	3.14	2.60	2.77		1.96	1.56	295	273	266		267			272	372	222	236	232	212	210		208	195
J.O.	16:0									34	33	36	35	36		34	33	39	37	36	39	37		33	35	12	11	15	16	14		11	11
male	16:1									6	6	5	5	5		5	5	1	2	2	2	2		2	2	6	5	6	6	6		6	5
age 59	18:0	n.e.		n.e.				n.e.		2	2	4	3	3		2	2	14	14	11	13	13		14	14	T	T	T	1	1		1	T
(IHD)	18:1									48	49	46	46	46		50	51	17	15	14	14	13		16	16	23	24	31	29	24		21	24
	18:2									9	9	9	9	9		8	9	17	18	22	20	21		21	20	50	50	40	40	48		54	51
	20:3									-	-	-	-	-		-	-	2	2	3	2	3		2	2	T	T	T	T	T		T	T
	20:4									1	T	T	T	1		1	T	10	11	11	10	11		12	11	9	9	7	8	8		8	9
		541	763	812	655	710	687	610	695	1.37	1.56	1.56	2.20	1.77	2.17	2.22	1.89	320		215	156	197	250	270		304		267	282	320			330
										Mean from 2 days								Mean from 2 days								Mean from 2 days							
R.McG.	16:0									26		30	27	30	30	28	26	33	31	36	33	33		33	31	11		12	12	10	10	11	10
male	16:1									9		8	7	7	6	7	9	2	2	1	1	1		2	2	8		9	8	7	8	8	8
age 54	18:0	n.e.		n.e.				n.e.		3		3	5	4	4	4	3	16	14	16	16	15		17	16	1		2	1	2	T	T	1
(IHD)	18:1									52		50	50	49	49	49	51	18	19	16	16	19		18	21	28		29	27	29	27	26	25
	18:2									8		8	10	10	9	10	10	17	19	16	18	17		17	16	43		42	44	44	47	46	47
	20:3									-		-	-	-	-	-	-	4	4	5	5	4		4	4	-		-	-	-	-	-	-
	20:4									1		1	1	1	2	2	2	10	11	10	11	10		9	10	9		7	9	8	8	9	9

Abbreviations: n.e. - not estimated
T - trace amount

The first position in the columns represents the concentration of total lipids in the serum: Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq/l.

APPENDIX TABLE 6a.

Short-term effects of HGH injection associated with fasting on percentage composition of serum lipid fatty acids.

(Expressed as percentage of total fatty acids in the fraction).

The short-term effects of HGH were measured 4 and 8 hours after the first HGH injection.

Subject	Fatty acid short hand design.	Free fatty acids							Triglyceride fatty acids							Phospholipid fatty acids							Cholesteryl fatty acids						
		8 a.m.		12 noon		4 p.m.		Next day	8 a.m.		12 noon		4 p.m.		Next day	8 a.m.		12 noon		4 p.m.		Next day	8 a.m.		12 noon		4 p.m.		Next day
		Pre-treatment	Before* injection	Pre-treatment	4 hours after injection	Pre-treatment	8 hours after injection	overnight fast	Pre-treatment	Before* injection	Pre-treatment	4 hours after injection	Pre-treatment	8 hours after injection	overnight fast	Pre-treatment	Before* injection	Pre-treatment	4 hours after injection	Pre-treatment	8 hours after injection	overnight fast	Pre-treatment	Before* injection	Pre-treatment	4 hours after injection	Pre-treatment	8 hours after injection	overnight fast
		523	629	463	1053	755	1305	880	1.48	1.53	1.07	1.30	1.35	1.69	1.34	314	310	282	220	290	290	266	271	272	257	290	237	279	268
W.G.	16:0	27	28	31	27	24	20	27	33	33	32	32	29	29	33	28	28	27	31	29	28	34	12	11	12	11	8	11	11
	16:1	3	2	3	5	4	4	5	7	6	7	8	6	8	6	1	1	1	1	1	1	1	5	6	6	6	4	5	6
	18:0	25	29	24	18	17	15	19	5	5	4	5	4	5	6	15	16	18	18	18	18	15	1	1	1	1	1	1	1
	18:1	33	29	33	42	45	52	41	45	45	47	48	49	49	47	22	22	22	22	23	22	19	30	30	31	31	32	34	31
	18:2	12	12	10	8	10	9	7	9	10	10	9	12	10	9	19	19	16	17	15	16	19	43	44	45	41	46	39	41
	20:3	T	T	T	T	T	T	T	T	T	T	T	T	T	T	3	2	5	2	3	2	2	T	1	T	T	T	T	T
	20:4	T	T	T	T	T	T	T	T	T	T	T	T	T	T	12	12	11	10	12	13	10	7	7	6	6	8	9	7
		950	1040	1026	1350	960	1748	960	0.96	1.62	0.85	1.30	1.44	1.84	1.50	250	275	238	220	244	275	224	170	163	184	176	158	189	163
W.L.	16:0	27	30	26	20	26	16	28	29	29	29	27	27	32	30	33	30	35	38	29	32	33	13	13	11	10	12	11	12
	16:1	4	4	6	4	4	4	4	6	6	6	6	6	6	6	1	1	1	1	1	1	1	8	7	5	6	7	6	6
	18:0	18	16	13	15	16	13	14	4	3	3	5	4	3	4	14	15	13	14	12	15	13	1	1	1	1	1	2	1
	18:1	44	43	48	55	46	58	47	53	52	52	52	52	50	51	21	21	17	20	17	20	19	30	30	30	31	31	32	33
	18:2	8	7	8	7	9	9	7	9	9	10	11	10	9	10	19	15	18	15	19	17	19	43	42	44	40	42	44	40
	20:3	T	T	T	T	T	T	T	T	T	T	T	T	T	T	1	3	4	3	4	3	3	T	T	T	2	1	T	T
	20:4	T	T	T	T	T	T	T	T	T	T	T	T	T	T	11	13	12	9	10	11	13	6	7	9	11	8	6	8
		700	564	600	710	700	1340	608	2.61	2.43	2.51	1.95	2.50	1.85	1.85	213	223	192	157	175	200	186	157	164	150	159	163	163	191
A.H.	16:0	25	28	32	28	37	26	32	33	35	37	34	32	33	36	30	30	36	42	37	36	38	14	13	14	16	14	15	15
	16:1	5	5	3	4	5	3	5	7	10	7	9	7	8	7	1	1	2	2	3	2	1	9	8	9	7	8	9	12
	18:0	23	18	14	13	11	12	16	5	5	3	4	5	2	5	18	18	17	16	17	18	19	1	1	1	1	1	1	1
	18:1	47	44	43	47	41	55	42	48	44	51	47	49	50	46	22	22	22	18	20	19	18	35	34	36	34	35	34	34
	18:2	6	5	6	6	4	5	6	7	5	3	6	7	6	7	13	13	11	11	10	11	15	35	36	36	36	42	33	32
	20:3	T	T	T	T	T	T	T	T	T	T	T	T	T	T	2	3	2	3	3	3	3	T	T	T	T	T	T	T
	20:4	T	T	T	T	T	T	T	T	T	T	T	T	T	T	13	12	10	11	11	11	10	6	8	6	6	6	8	7

Abbreviations: T - trace amount

* Day of experiment.

The first position in the columns represents the concentration of total lipids in the serum: Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acid concentration in μEq/l.

APPENDIX TABLE 6b.

The long-term effects of HGH injections on the percentage composition of serum lipid fatty acids.

(Expressed as percentage of total fatty acids in the fraction)

The long-term effects were measured 24 hours after each of the two HGH injections.

Subject	Fatty acid short hand design.	Free fatty acids					Triglyceride fatty acids					Phospholipid fatty acids					Cholesteryl fatty acids				
		Pre-treatment		On treatment days		After last injection days	Pre-treatment		On treatment days		After last injection days	Pre-treatment		On treatment days		After last injection days	Pre-treatment		On treatment days		After last injection days
				1	2	2			1	2	2			1	2	2			1	2	2
		522	629	880	495	425	1.48	1.53	1.34	1.35	1.16	314	310	266	196	290	271	272	268	288	282
W.G.	16:0	27	28	27	31	31	33	33	33	30	30	28	28	34	27	28	12	11	13	11	13
male age	16:1	3	2	5	4	2	7	6	6	6	6	1	1	1	1	1	5	6	6	5	5
45	18:0	25	29	19	18	24	5	5	6	5	7	15	16	15	16	17	1	1	1	1	1
	18:1	33	29	41	41	35	45	45	47	48	47	22	22	19	19	21	30	30	31	36	29
	18:2	12	12	7	6	8	9	10	9	10	10	19	19	19	20	20	43	44	41	40	43
	20:3	T	T	T	T	T	T	T	T	T	T	3	2	2	4	2	T	1	T	T	T
	20:4	T	T	T	T	T	T	T	T	T	T	12	12	10	13	9	7	7	7	6	7
		950	1040	960	830	810	0.96	1.62	1.50	1.26	1.64	250	275	224	146	201	170	163	190	195	193
W.L.	16:0	27	30	28	28	26	29	29	30	31	31	33	30	33	35	31	13	13	12	13	14
male age	16:1	4	4	4	5	5	6	6	6	5	5	1	1	1	1	1	8	7	6	6	6
65	18:0	18	16	14	12	13	4	3	4	3	4	14	15	13	13	14	1	1	1	1	1
	18:1	44	43	47	50	48	53	52	51	50	50	21	21	19	17	18	30	30	33	31	30
	18:2	8	7	7	5	7	9	9	10	10	10	19	17	19	19	19	43	42	40	42	42
	20:3	T	T	T	T	T	T	T	T	T	T	1	3	3	3	4	T	T	T	T	T
	20:4	T	T	T	T	T	T	T	T	T	T	11	13	13	12	12	6	7	8	7	7
		700	564	600	628	300	2.61	2.43	1.85	1.95	1.85	213	223	186	160	220	157	164	191	206	175
A.H.	16:0	25	28	32	32	37	33	35	36	34	33	30	30	38	28	35	14	13	15	17	14
male age	16:1	5	5	5	4	8	7	10	7	10	8	1	1	1	1	1	9	8	12	7	6
48	18:0	23	18	16	16	15	5	5	5	4	3	18	18	19	16	16	1	1	1	1	1
	18:1	47	44	42	43	34	48	44	46	47	50	22	22	18	22	17	35	34	34	34	31
	18:2	6	5	6	4	5	7	5	7	6	6	15	15	15	19	17	35	36	32	34	41
	20:3	T	T	T	T	T	T	T	T	T	T	2	3	3	3	3	T	T	T	T	T
	20:4	T	T	T	T	T	T	T	T	T	T	13	12	10	12	11	6	8	7	6	7

Abbreviations: T - trace amount

The first position in the columns represents the concentration of total lipids in the serum: Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq/l.

APPENDIX TABLE 7.

The percentage composition of serum lipid fatty acids in acromegalic and normal women (age 50-60).

(Expressed as percentage of total fatty acids in the fraction)

Fatty acid short hand design.	Free fatty acids							Triglyceride fatty acids							Phospholipid fatty acids							Cholesteryl fatty acids						
	Normal subjects				Acromegaly			Normal subjects				Acromegaly			Normal subjects				Acromegaly			Normal subjects				Acromegaly		
	590	555	655	680	354	210	600	1.32	2.38	1.09	1.93	1.60	1.98	1.93	n.e.	n.e.	250	270	n.e.	n.e.	266	321	238	248	246	250	235	186
16:0	26	22	29	28	32	23	28	33	30	31	27	34	36	36	33	29	29	32	32	32	33	13	12	13	9	12	12	12
16:1	4	4	5	5	4	2	3	5	8	6	4	5	5	6	1	1	2	2	1	1	1	6	8	6	4	5	5	8
18:0	15	18	17	11	20	28	23	3	3	6	2	5	7	6	16	17	17	17	14	18	16	T	1	1	1	1	1	1
18:1	44	48	42	43	33	37	39	48	47	49	43	43	44	45	15	18	20	11	15	17	21	24	26	26	15	20	26	30
18:2	9	8	7	13	10	11	7	10	10	7	24	12	9	7	18	20	22	26	25	21	19	48	46	47	62	57	49	45
20:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4	1	2	2	2	1	-	-	-	-	-	-	-
20:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	11	9	10	9	7	9	9	7	7	9	5	7	4
Sum of saturated acids	41	40	46	38	52	51	51	36	33	37	29	39	43	42	49	46	46	49	46	50	49	13	13	14	10	13	13	13
Sum of monounsaturated acids	48	52	47	48	37	39	42	53	55	55	47	37	39	42	16	19	23	13	16	18	22	30	34	32	19	25	31	38

The statistically significant differences from these data are presented in Tables 15, 17, 19 and 21.

Abbreviations: n.e. - not estimated

The first position is the columns represents the concentration of total lipids in the serum: Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in µEq/l.

APPENDIX TABLE 8.

The immediate effects of noradrenaline infusion on the percentage composition*of serum lipid fatty acids (After a 15-minute infusion of noradrenaline).

Subjects	Fatty acid short hand design.	Free fatty acids		Triglyceride fatty acids		Phospholipid fatty acids		Cholesteryl fatty acids	
		Before infusion	After infusion	Before infusion	After infusion	Before infusion	After infusion	Before infusion	After infusion
		226	465	1.16	1.31	272	255	229	237
M.D.	16:0	27	21	28	28	31	27	12	12
male	16:1	4	3	7	6	2	1	3	4
age 29	18:0	20	17	5	5	16	17	1	1
healthy	18:1	42	50	49	49	16	15	23	25
	18:2	7	9	12	12	22	20	47	52
	20:3	-	-	-	-	1	1	-	-
	20:4	-	-	-	-	12	18	7	7
		672	1111	1.03	1.40	n.e.	n.e.	287	265
R.O.	16:0	29	26	32	23	42	38	11	12
male	16:1	5	6	5	4	1	1	2	2
age 38	18:0	14	11	4	3	16	15	1	1
healthy	18:1	46	49	50	58	15	17	32	30
	18:2	6	8	8	11	17	17	47	44
	20:3	-	-	-	-	2	1	-	-
	20:4	-	-	-	-	1	11	8	10
		743	845	1.82	1.67	345	279	289	249
E.L.	16:0	27	24	32	29	36	32	11	10
male	16:1	4	5	5	6	3	2	5	5
age 64	18:0	16	12	3	4	15	16	1	1
hypertensive	18:1	47	52	49	50	19	19	27	27
	18:2	6	7	10	11	18	19	49	50
	20:3	-	-	-	-	3	3	-	-
	20:4	-	-	-	7	6	9	7	7
		618	651	1.75	1.60	n.e.	n.e.	550	540
T.A.	16:0	32	31	25	25	39	27	12	11
male	16:1	5	5	5	5	1	1	4	2
age 22	18:0	15	14	4	4	16	17	21	21
hypercholesterol-aemia	18:1	41	43	43	43	15	16	24	23
	18:2	7	7	13	12	20	26	51	50
	20:3	-	-	-	-	1	1	-	-
	20:4	-	-	-	-	9	12	9	13
		239	677	2.19	2.93	292	352	317	311
G.I.	16:0	26	25	28	35	31	28	12	13
male	16:1	4	5	6	5	1	1	6	7
age 43	18:0	18	15	4	3	17	17	1	1
hypertensive	18:1	42	45	50	46	19	20	25	25
	18:2	8	10	12	10	21	22	51	48
	20:3	-	-	-	-	2	2	-	-
	20:4	-	-	-	-	8	10	6	6
		709	1318	0.72	0.77	260	150	174	165
A.U.	16:0	29	29	28	30	33	26	14	14
male	16:1	5	6	6	6	2	1	7	6
age 47	18:0	15	11	4	3	17	16	1	1
hypertensive	18:1	44	46	50	52	19	18	30	30
	18:2	6	9	12	10	22	22	45	44
	20:3	-	-	-	-	1	6	-	-
	20:4	-	-	-	-	6	10	3	5

Abbreviations: n.e. - not estimated

* Expressed as percentage of total fatty acids in the fraction.

The first position in the columns represents the concentration of total lipids in the serum:
Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq/l.

APPENDIX TABLE 9.

Effects of prolongation of overnight fast by 8 hours on the percentage composition of serum lipid fatty acids.

(Expressed as percentage of total fatty acids in the fraction)

	Fatty acid short hand design.	SUBJECTS A F1	A F2	B F1	B F2	C F1	C F2	D F1	D F2	E F1	E F2	F F1	F F2	G F1	G F2	H F1	H F2	I F1	I F2	J F1	J F2	K F1	K F2	L F1	L F2	Mean of 12 subjects F_1	Mean of 12 subjects F_2
		625	670	1218	1409	400	855	655	810	530	640	520	770	523	755	950	960	700	700	655	1010	680	1380	330	1210		
Free fatty acids	16:0	27	28	23	22	26	27	32	32	26	28	14	15	27	24	27	26	25	37	32	28	28	27	9	23	25	26
	16:1	5	6	5	5	5	5	8	9	5	6	2	2	3	4	4	4	5	5	7	5	5	4	1	3	5	5
	18:0	19	12	13	11	13	11	12	10	14	14	22	17	25	17	18	16	23	11	14	13	11	15	31	15	18	14
	18:1	39	43	49	53	45	46	45	45	38	42	52	55	33	45	44	46	47	41	35	41	43	44	48	49	43	46
	18:2	10	10	11	9	11	11	6	6	12	8	11	11	12	10	8	9	6	4	9	9	14	11	13	9	11	9
		1.50	n.e.	1.90	1.70	1.31	n.e.	1.40	n.e.	1.30	n.e.	1.58	2.20	1.48	1.35	0.90	1.44	2.61	2.60	0.99	0.58	1.07	1.79	1.38	0.77		
Triglyceride fatty acids	16:0	29	31	24	26	28	27	32	32	34	28	31	28	33	29	29	27	33	32	29	34	31	31	24	28	29	29
	16:1	5	5	7	7	6	4	8	9	4	3	6	4	7	6	6	6	7	7	6	4	7	6	5	4	7	5
	18:0	4	3	3	2	3	3	4	2	6	5	5	4	5	4	4	4	5	5	4	2	4	2	5	4	4	3
	18:1	50	52	52	52	50	47	48	50	39	47	45	48	55	49	53	52	48	49	45	44	43	45	58	51	48	49
	18:2	11	14	13	13	14	16	8	8	17	17	16	15	9	12	9	10	7	7	16	10	15	14	9	14	11	12
		n.e.	n.e.	322	404	412	n.e.	n.e.	n.e.	n.e.	n.e.	337	335	314	290	250	244	213	175	n.e.	n.e.	337	324	256	n.e.		
Phospholipid fatty acids	16:0	26	30	32	38	39	39	29	28	34	32	29	34	28	29	33	29	30	37	29	34	33	35	18	28	30	33
	16:1	1	2	2	2	1	1	3	2	2	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	18:0	16	16	16	15	14	14	14	15	16	16	16	14	15	18	14	12	18	17	13	15	16	17	18	16	16	15
	18:1	20	18	16	16	13	12	19	19	14	15	14	14	22	23	21	17	22	20	13	14	12	11	18	16	17	16
	18:2	24	21	23	19	16	17	20	21	23	20	26	23	19	15	19	19	15	10	29	25	24	21	26	23	22	19
	20:3	2	2	2	2	3	2	1	4	2	2	2	2	3	3	1	4	2	3	2	2	3	3	3	3	2	3
	20:4	11	11	8	7	13	15	10	9	10	12	12	11	12	12	11	10	13	11	19	10	11	11	13	14	11	11
		n.e.	n.e.	445	440	260	259	n.e.	n.e.	250	293	258	279	271	237	170	158	157	163	292	250	246	246	184	185		
Cholesteryl	16:0	9	10	12	11	12	13	11	11	16	14	8	11	12	8	13	12	14	14	8	10	11	10	11	12	11	11
	16:1	3	4	5	6	5	4	3	2	3	3	3	3	5	4	8	7	9	8	3	4	3	3	3	4	4	5
	18:0	2	1	1	1	T	1	2	2	1	1	1	1	1	1	1	1	1	1	T	T	1	1	1	1	1	1
	18:1	23	22	22	20	26	25	24	26	25	25	25	25	30	32	30	31	35	35	17	17	17	18	26	26	25	25
	18:2	56	55	56	56	48	45	43	43	48	49	52	52	43	46	43	42	35	42	65	65	63	62	52	52	50	50
	20:4	6	9	4	5	9	12	6	8	7	7	7	8	7	8	6	8	6	6	6	4	5	6	7	5	6	7

Abbreviations: F_1 - 15 hours of fasting (overnight fast)
F_2 - 23 hours of fasting
n.e. - not estimated
T - trace amount

Subjects E-F: Ischaemic heart disease, age 38-50
Subjects G-I: Convalescents after renal colic, age 45-65
Subjects J-L: Healthy, age 27-50.

The first position represents the concentration of total lipids in the serum:
Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq/l.

The statistically significant changes are presented in Tables 14, 16, 18 and 20.

APPENDIX TABLE 10.

Effects of acute myocardial infarction on the percentage composition of serum lipid fatty acids.

(Expressed as percentage of total fatty acids in the fraction).

Subjects	Fatty acid short hand design.	Free fatty acids			Triglyceride fatty acids			Phospholipid fatty acids			Cholesteryl fatty acids		
		1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day
		1040	960	860	0.97	0.88	1.40	230	247	n.e.	250	229	n.e.
A.B.	16:0	31	26	28	31	30	29	29	32	36	11	12	12
male	16:1	5	8	7	7	5	7	2	1	2	5	4	2
age 48	18:0	13	15	15	3	4	6	16	14	15	T	T	1
M.I.	18:1	44	49	44	47	47	47	15	14	14	19	21	22
Survived.	18:2	7	7	7	11	13	11	22	24	20	60	55	57
	20:3	-	-	-	-	-	-	3	3	3	-	-	-
	20:4	-	-	-	-	-	-	13	12	11	6	8	6
		1210	915	850	n.e.	0.54	n.e.	443	480	n.e.	n.e.	258	n.e.
J.G.	16:0	30	29	25	35	31	32	39	40	37	11	10	14
male	16:1	3	6	4	4	7	6	2	3	2	6	5	6
age 59	18:0	13	11	14	3	2	2	13	12	13	T	T	1
M.I.	18:1	44	45	44	43	45	48	16	16	16	22	20	31
Survived.	18:2	8	7	7	11	13	10	16	16	16	51	49	44
	20:3	-	-	-	-	-	-	1	2	1	-	-	-
	20:4	-	-	-	-	-	-	15	16	15	10	16	15
		766	592	n.e.	n.e.	1.35	1.52	n.e.	289	214	n.e.	217	207
J.H.	16:0	26	24	29	27	27	25	38	36	37	11	12	12
male	16:1	6	4	3	7	5	5	1	1	2	5	5	5
age 57	18:0	13	13	11	3	4	3	14	15	15	T	1	1
M.I.	18:1	48	52	50	51	53	53	16	15	14	29	27	28
Survived.	18:2	7	7	6	11	12	13	19	18	18	50	51	48
	20:3	-	-	-	-	-	-	2	3	3	-	-	-
	20:4	-	-	-	-	-	-	9	11	11	5	5	4
		854	780	n.e.	1.38	1.76	n.e.	n.e.	65	n.e.	185	199	n.e.
J.S.	16:0	27	28		32	32		43	40		11	12	
male	16:1	4	2		6	6		2	1		6	6	
age 60	18:0	15	12		3	4		13	15		1	1	
M.I.	18:1	48	46		50	47		17	18		29	33	
Survived.	18:2	7	9		8	12		17	18		46	46	
	20:3	-	-		-	-		1	1		-	-	
	20:4	-	-		-	-		7	8		6	2	
		1242	800	n.e.	2.00	1.42	n.e.	n.e.	n.e.	n.e.	215	209	n.e.
J.C.	16:0	24	21		27	29		31	36		10	12	
male	16:1	4	3		5	5		2	2		5	5	
age 45	18:0	14	15		3	7		15	13		T	T	
M.I.	18:1	49	52		54	52		19	18		26	28	
Survived.	18:2	10	9		11	9		20	20		51	48	
	20:3	-	-		-	-		2	2		-	T	
	20:4	-	-		-	-		11	9		7	6	
		n.e.	n.e.	n.e.	1.58	2.03	n.e.	212	n.e.	n.e.	197	169	n.e.
J.C.	16:0	30	31		29	30	34	37	37		12	11	11
male	16:1	5	5		7	9	7	1	2		5	6	4
age 60	18:0	12	12		5	3	7	16	15		1	1	1
M.I.	18:1	45	42		48	51	45	16	18		24	29	29
Survived.	18:2	8	10		12	8	8	20	17		51	45	48
	20:3	-	-		-	-	-	2	2		-	-	-
	20:4	-	-		-	-	-	9	9		7	8	7
		704	746	552	0.97	1.40	1.24	n.e.	n.e.	n.e.	290	257	250
J.M.	16:0	22	29	24	26	33	29	23	34	28	11	14	15
male	16:1	5	5	5	5	7	5	1	1	1	5	5	6
age 58	18:0	14	11	15	3	3	3	21	16	15	2	1	T
(cerebral vascular episode	18:1	54	47	49	49	51	50	20	20	23	30	31	30
with haemiplegia between the	18:2	6	8	6	17	7	12	21	17	23	44	45	43
1st and 2nd day	20:3	-	-	-	-	-	-	3	2	3	-	-	-
Survived.	20:4	-	-	-	-	-	-	12	9	8	8	5	6
		684	832	908	n.e.	n.e.	1.58	n.e.	n.e.	47	n.e.	n.e.	154
J.C.	16:0	23	31	28	29	32	16	37	38	41	13	12	12
female	16:1	4	3	5	8	6	5	1	1	1	6	6	5
age 57	18:0	11	13	12	4	3	2	17	16	16	1	1	1
M.I.	18:1	50	44	48	49	49	40	16	15	15	30	27	31
Sudden death	18:2	11	10	8	11	11	32	19	18	16	43	47	45
on 3rd day.	20:3	-	-	-	-	-	-	3	3	1	-	1	-
	20:4	-	-	-	-	-	5	8	9	9	7	7	7
		2150	832	642	n.e.	2.01	2.27	197	198	n.e.	n.e.	264	273
R.I.	16:0	27	22	25	29	26	27	34	30	30	12	14	11
female	16:1	6	6	7	8	6	8	1	2	2	8	8	7
age 62	18:0	7	12	6	3	4	5	19	15	17	1	1	1
Ischaemic chest pain	18:1	54	56	56	51	52	55	16	19	23	30	29	30
No M.I.	18:2	6	7	6	8	11	12	18	19	18	45	42	46
Survived.	20:3	-	-	-	-	-	-	3	4	3	-	-	-
	20:4	-	-	-	-	-	-	9	11	8	5	5	8

Abbreviations: n.e. - not estimated
T - trace amount

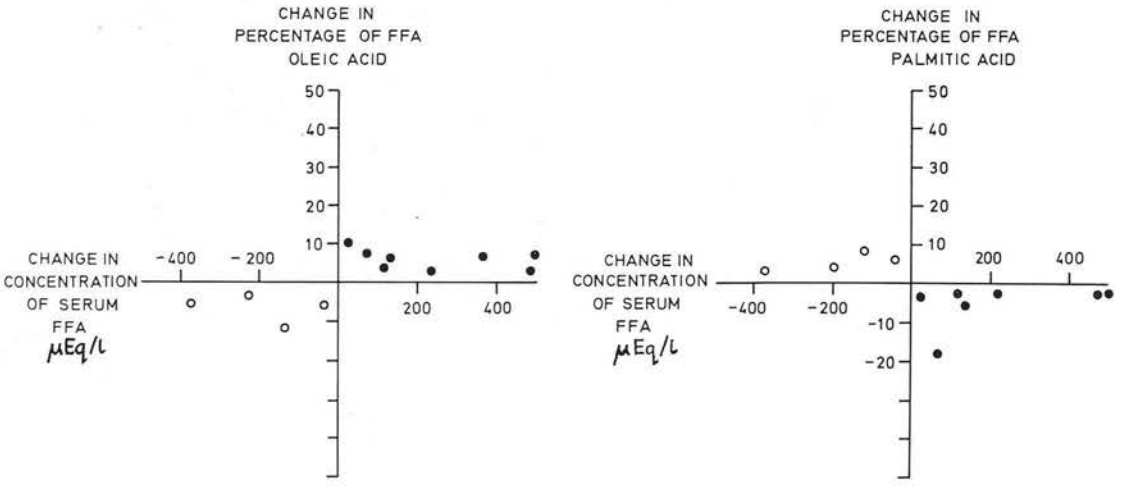
The first position in the columns represents the concentration of total lipids in the serum:
Total cholesterol concentrations in mg./100 ml.
Total phospholipid concentrations in mg./100 ml.
Total triglyceride concentrations in mmoles/l.
Total free fatty acids in μEq./l.

Appendix Fig. 1 The correlation between changes in concentration of serum FFA and changes in percentage of FFA oleic and palmitic acids in response to treatment with thyroid hormones and antithyroid therapy

Hypothyroid subjects treated with thyroid hormones

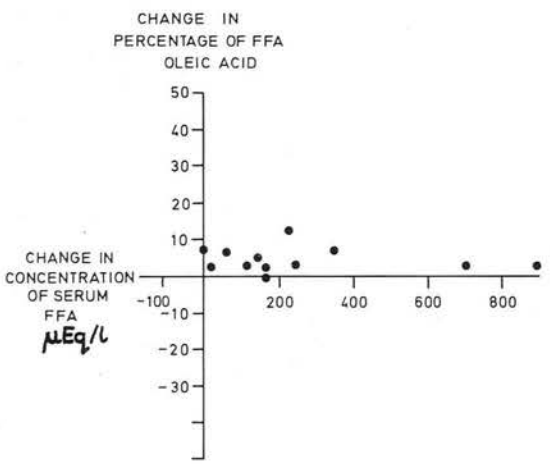
Hyperthyroid subjects treated with antithyroid therapy

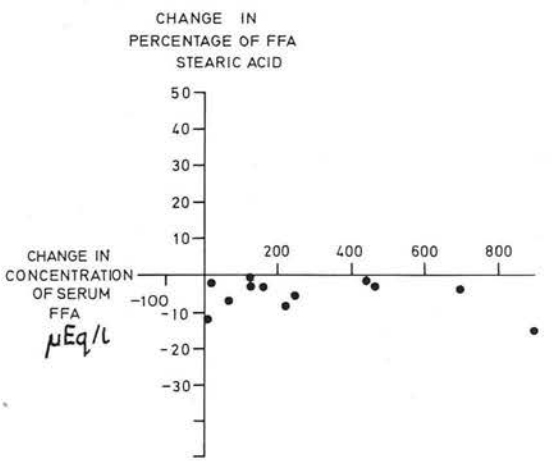
THYROID HORMONE ACTIVITY



Appendix Fig. 2 The correlation between changes in concentration of serum FFA and changes in percentage of FFA oleic and stearic acids in response to prolongation of overnight fast by 8 hours

FASTING

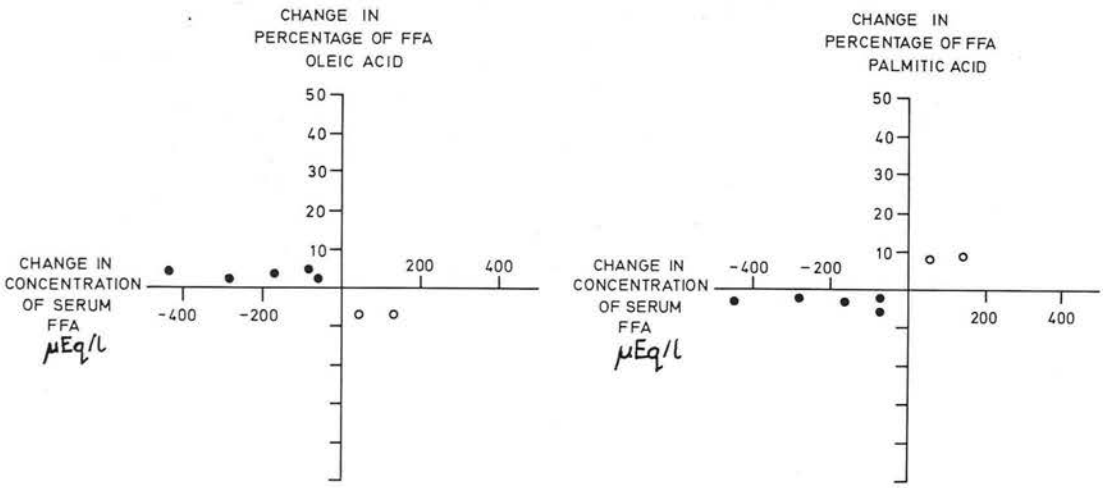

CHANGE IN
PERCENTAGE OF FFA
OLEIC ACID
CHANGE IN
CONCENTRATION
OF SERUM
FFA
µEq/l
50
40
30
20
10
-100
-10
-20
-30
200
400
600
800


CHANGE IN
PERCENTAGE OF FFA
STEARIC ACID
CHANGE IN
CONCENTRATION
OF SERUM
FFA
µEq/l
50
40
30
20
10
-100
-10
-20
-30
200
400
600
800

Appendix Fig. 3 The correlation between changes in concentration of serum FFA and changes in percentage of FFA oleic and palmitic acids observed between the first and the second day after myocardial infarction

o Patients severely ill
(one died on the third day and the second developed a cerebral vascular episode with hemiplegia on the second day)

MYOCARDIAL INFARCTION



B I B L I O G R A P H Y

ABELL, L.L., BRODIE, B.B., and KENDALL, F.E., (1952) J.biol.Chem., 195, 357.

AFTERGOOD, L., and ALFIN-SLATER, R.B., (1965) J.Lipid Res., 6, 287.

AFTERGOOD, L., and ALFIN-SLATER, R.B., (1967) J.Lipid Res., 8, 126.

ALBERS, H.J., and GORDON, S., (1962) Proc.Soc.exp. Biol.Med., 109, 860.

ALBRINK, M.J., and MAN, E.B., (1959) Arch .intern.Med., 103, 4.

ALFIN-SLATER, R.B., and JORDAN, P., (1960) Am.J.clin.Nutr., 8, 325.

ALLEN, R.J.L., (1940) Biochem.J., 34, 853.

ANSELL, G.B., and HAWTHORNE, J.N., (1964) "Phospholipids", Elsevier Publishing Company: Amsterdam - London - New York. p.189.

ANTONIS, A., and BERSOHN, I., (1960) Lancet, 1, 998.

ARLOW, J.A., (1945) Psychosomat.Med., 7, 195. (quoted by Carlson et al. 1965)

BAKER, W.R., THOMSON, R.H.S., and ZILKHA, K.J. (1964) J.Neurol.Neurosurg. Psychiat., 27, 408.

BAKER, W.R., THOMSON, R.H.S., and ZILKHA, K.J. (1966) J.Neurol.Neurosurg. Psychiat., 29, 95.

BASSET, T., and WALLACE, A.L.C., (1966) Metabolism, 15, 933.

van BEERS, G.J., de IONGH, H., and BOLDINGH, J., (1958) in: Proc. 4th Intern. Conf.Lipids "Essential Fatty Acids". ed. H.M. Sinclair, Butterworths (London), p.43.

BERRY, G., MOXHAM, A., SMITH, I.E., KELLIE, A.E., and NABARRO, J.D.N., (1963) J.Atheroscler.Res., 3, 380.

BJØNSTAD, P., and BREMER, J., (1966) J.Lipid Res., 7, 38.

BOGDONOFF, M.D., ESTES, E.H. Jr., and TROUT, D., (1959) Proc.Soc.exp.Biol.Med., 100, 503.

BORGSTRÖM, B., (1952) Acta physiol.scand., 25, 111.

BORGSTRÖM, B., and OLIVECRONA, T., (1961) J.Lipid Res., 2, 263.

BÖTTCHER, C.J.F., BOELSMA-VAN HOUTE, E., TER HAAR ROMENY-WACHTER, C.C., WOODFORD, F.P., van GENT, C.M., (1960) Lancet, 2, 1162.

BÖTTCHER, C.J.F., and WOODFORD, F.P., (1961) J.Atheroscler.Res., 1, 434.

BÖTTCHER, C.J.F., and WOODFORD, F.P., (1962) Fedn.Proc.Fedn.Am.Socs.exp.Biol., 21, Suppl. II, 15.

BÖTTCHER, C.J.F., WOODFORD, F.P., BOELSMA-VAN HOUTE, E., and van GENT, C.M., (1959) Recl.Trav.chim.Pays-Bas Belg., 78, 794.

BOYD, G.S., (1963) in:Biochem.Soc.Symp. 24, "The Control of Lipid Metabolism", Ed.:J.K. Grant. Academic Press, London. pp.79-98.

BROWN, D.F., KINCH, S.H., and DOYLE, J.T., (1965) New Engl.J.Med., 273, 947.

BROWN, W.R., and HANSEN, A.L., (1937) Proc.Soc.exp.Biol.Med., 36, 113.

BUCHFIELD, H.P., and STORRS, E.E., (1962) "Biochemical Application of Gas Chromatography," Academic Press, New York - London.

BYERS, S.O., and FRIEDMAN, M., (1960) Am.J.Physiol., 198, 629.

CAREN, R., and CORBO, L., (1966) J.clin.Endocr.Metab., 26, 470.

CARLSON, L.A., (1963) J.Atheroscler.Res., 3, 334.

CARLSON, L.A., BOBERG, J., and HOGSTEDT, B., (1965) in: "Handbook of Physiology" - Section 5 "Adipose Tissue". Section Ed.: A.E. Renaud and C.F. Cahill, Jr., American Physiological Society, Washington, Waverley Press, Baltimore pp. 625-644.

CARLSON, L.A., and EKELUND, L.G., (1963) J.clin.Invest., 42, 714.

CARLSON, L.A., EKELUND, L.G., and ORO, L., (1963) J.Lab.clin.Med., 61, 724.

CARLSON, L.A., and PERNOW, B., (1959) J.Lab.clin.Med., 53, 833.

CARROLL, K.K., (1960) J.Lipid Res., 2, 135.

CHALMERS, T.M., (1964) Post-grad.med.J., 40, 464.

CHALMERS, T.M., PAWAN, G.L.S., and KEKWICK, A., (1960) Lancet, 2, 6.

CHOBANIAN, A.V., and HOLLANDER, W., (1962) J.clin.Invest., 41, 1732.

COCHRAN, B., MARBACH, J.E.P., and POUCHER, R.L., (1965) Clin.Res., 13, 130.

COLLINS, F.D., (1960) Nature Lond., 188, 297.

The COOPERATIVE STUDY OF LIPOPROTEIN AND ATHEROSCLEROSIS, (1956) Circulation, 14, 691.

CORNWELL, D.G., KRUGER, F.A., HAMWI, G.J., and BROWN, J.B., (1962) Metabolism, 11, 840.

DANOWSKI, T.S., BONESSI, J.V., SARVER, M.E., MOSES, C. (1964) Metabolism 13 739

DEBONS, A.F., and SCHWARTZ, I.L., (1961) J.Lipid Res., 2, 86.

DEYKIN, D., and VAUGHAN, M., (1963) J.Lipid Res., 4, 200.

DOLE, V.P., (1956) J.clin.Invest., 35, 150.

DOLE, V.P., JAMES, A.T., WEBB, J.P.W., RIZACK, M.A. and STURMAN, M.F., (1959) J.clin.Invest., 38, 1544.

DOYLE, J.T., HESLIN, S.H., HILLEBOE, H.F., FORMEL, P.F., KORNS, R.F., (1957) Amer.J.publ.Hlth., 47, 25.

DURY, A., (1955) Proc.Soc.exp.Biol.Med., 89, 508.

DURY, A., and TREADWELL, C.R., (1958) Proc.Soc.exp.Biol.Med., 97, 150.

DUSTIN, J.P., FREDRICKSON, D.S., LAUDAT, P., and ONO, K., (1961) Fedn. Proc.Fedn.Am.Socs.exp.Biol., 20, 270.

ECKSTEIN, H.C., (1925) J.biol.Chem., 64, 797.

ELLEFSON, R.D., and MASON, H.L., (1964) Endocrinology, 75, 179.

EPSTEIN, F.H., BROCK, W.H., HANDE, A., and FRANCIS, T., (1959) Amer.J.Med., 24, 39.

EVANS, J.R., (1964a) Can.J.Biochem.Physiol., 42, 955.

EVANS, J.R., (1964b) Circulation Res. Suppl. II - 96.

EVANS, J.R., HOLLENBERG, C.H., and ANGEL, A., (1963) Fedn.Proc.Fedn.Am.Socs. exp.Biol., 22, 376.

EVANS, J.D., WALLDRON, J.M., OLEKYSHYN, N.L., and RIEMENSCHNEIDER, R.W., (1956), J.biol.Chem., 218, 255.

FEIGELSON, E.B., PFAFF, W.W., KARMEN, A., and STEINBERG, D., (1961) J.clin. Invest., 40, 2171.

FELDMAN, E.B., and CARTER, A.C., (1963) Metabolism, 12, 1132.

FLETCHER, R.F., and GLOSTER, J., (1964) J.clin.Invest., 43, 2104.

FLOCK, E.V., BOLLMAN, J.L., and BERKSON, J., (1948) Am.J.Physiol., 155, 402.

FOLCH, J., LEES, M., and SLOANE-STANLEY, G.H., (1957) J.biol.Chem., 226, 497.

FREDRICKSON, D.S., ALTROCCHI, D.H., AVIOLI, L.V., GOODMAN, De W.S., and GOODMAN, H.C., (1961) Ann.Intern.Med., 55, 1016.

FREDRICKSON, D.S., and GORDON, R.S., (1958) Physiol.Rev., 38, 585.

FREDRICKSON, D.S., and GORDON, R.S.,(1958), J.clin.Invest., 37, 1504.

FREDRICKSON, D.S., LEVY, R.I., and LEES, R.S., (1967a) New.Engl.J.Med., 276, 32.

FREDRICKSON, D.S., LEVY, R.I., and LEES, R.S., (1967b) New.Engl.J.Med., 276, 94.

FREDRICKSON, D.S., LEVY, R.I., and LEES, R.S., (1967c) New.Engl.J.Med., 276, 148.

FREDRICKSON, D.S., LEVY, R.I., and LEES, R.S., (1967d) New.Engl.J.Med., 276, 215.

FREDRICKSON, D.S., LEVY, R.I., and LEES, R.S., (1967e) New Engl.J.Med., 276, 273.

FRIEDMAN, M., and BYERS, S.O., (1955) J.clin.Invest., 34, 1369.

FRIEDMAN, M., GEORGE, S.S.T., BYERS, S.O., and ROSENMAN, R.H., (1960) J.clin.Invest., 39, 758.

GEYER, P., BENNET, A., and ROHR, A., (1962) J.Lipid Res., 3, 380.

GIDEZ, L.I., ROHEIN, P.S., and EDER, H.A., (1967) J.Lipid Res., 8, 7.

GLEMNON, A.J., BRECH, W.J., GORDON, E.S., (1967) Metabolism, 16, 503.

GLOMSET, J.A., PARKER, F., TJADEN, M., WILLIAMS, R.H., (1962) Biochim. biophys. Acta, 58, 398.

GLOMSET, J.A., (1962) Biochim.biophys.Acta, 65, 128.

GLOMSET, J.A., (1963) Biochim.biophys. Acta, 70, 389.

GOLDFIEN, A., and HAVEL, R.J., (1959) J.clin.Invest., 38, 1007.

GOLDRICK, R.B., and HIRSH, J., (1964) J.clin.Invest., 43, 1793.

GOODMAN, De W.S., (1958) J.Am.chem.Soc., 80, 3892.

GOODMAN, De W.S., (1964) J.clin.Invest., 43, 2026.

GOODMAN, De W.S., (1965) Physiol.Rev., 45, 747.

GOODMAN, De W.S., DEYKIN, D., and SHIRATORI, T., (1964) J.biol.Chem., 239, 1335.

GOODMAN, De W.S., and SHIRATORI, T., (1964) J.Lipid Res., 5, 578.

GORANSSON, G., (1965a) Acta physiol.scand., 64, 1.

GORANSSON, G., (1965b) Acta physiol.scand., 64, 204.

GORANSSON, G., (1965c) Acta physiol.scand., 64, 269.

GORANSSON, G., and OLIVECRONA, T., (1964) Acta physiol.scand., 62, 224.

GORANSSON, G., and OLIVECRONA, T., (1965) Acta physiol.scand., 63, 121.

GORDON, R.S.Jr., (1957) J.clin.Invest., 36, 810.

GORDON, R.S., and CHERKES, A., (1956) J.clin.Invest., 35, 206.

GRASSO, S., MICHAELS, G.D., KINSELL, L.W., (1964) Metabolism, 13, 1333.

GRASSO, S., MICHAELS, G.D., COELHO, M., and KINSELL, L.W., (1963) Circulation, 28, 657.

GREENBAUM, A.L., and McLEAN, P., (1953) Biochem.J., 54, 407.

GUNNING, G.B., MICHAELS, G., NEUMAN, L., SPLITTER, R., and KINSELL, L., (1963) J.Nutr., 79, 85.

HALLGREEN, B., STEINHAGEN, S., SVANBORG, A., and SVENNERHOLM, L., (1960) J.clin.Invest., 39, 1424.

HAMILTON, J.G., MUDREY, J.E., MILLER, O.N., and GOLDSMITH, G.A., (1961) Am.J.clin.Nutr., 9, 248.

HARLAN, W.R., LASZLO, J., BOGDONOFF, M.P., and ESTES, E.H., (1963) J.clin.Endocr.Metab., 23, 33.

HARPER, P.V., NEAL, W.B., and HLAVACEK, G.R., (1965) Metabolism, 2, 69.

HAVEL, R.J., and CARLSON, L.A., (1962) Biochem.J., 85, 39.

HAVEL, R.J., and GOLDFIEN, D., (1959) J.Lipid Res., 1, 102.

HAVEL, R.J., and GOLDFIEN, D., (1961) J.Lipid Res., 2, 389.

HIRSCH, J., and AHRENS, E.H., (1958) J.biol.Chem., 233, 311.

HOLLENBERG, C.H., and ANGEL, A., (1963) Am.J.Physiol., 205, 909.

HOLLENBERG, C.H., and DOUGLAS, D.E., (1962) Nature, Lond., 193, 1074.

HORNING, E.C., AHRENS, E.H., Jr., LIPSKY, S.R., MATTSON, F.H., MEAD, J.F., TURNER, D.A., and GOLDWATER, W.H., (1964) J.Lipid Res., 5, 20.

HUNTER, W.M., and GREENWOOD, F.C., (1962) Biochem.J., 85, 39.

IKELS, K.G., (1961) U.S.A.F., 61 - 108, p.1

IMAICHI, K., FUKUDA, J.I., NOTOMI, A., (1966) J.Biochem. (Tokyo), 59, 189.

de IONGH, H., and van PELT, J.G., (1962) J.Lipid Res., 3, 385.

JAECLE, J., (1902) Hoppe-Seyler's Z.physiol.Chem., 36, 53.

JAMES, A.T., (1957) Lancet, 1, 705.

JAMES, A.T., and MARTIN, A.J.P., (1952), Biochem.J., 50, 679.

JEANRENAUD, B., (1965) in "Handbook of Physiology" - Section 5: "Adipose Tissue". Section Ed. A.E. Renauld and C.F. Cahill, Jr., American Physiological Society, Washington; Waverley Press, Baltimore, pp. 169-177.

JURAND, J., and ALBERT-RECHT, F., (1962) Clin.chim.Acta, 7, 522.

JURAND, J., and OLIVER, M.F., (1963) J.Atheroscler.Res., 3, 547.

KABAL, J., and RAMEY, E.R., (1965) Proc.Soc.exp .Biol.Med., 119, 708.

KANNEL, W.B., DAUBER, T.R., KAGAN, A., REVOTSKIE, N., and STOKES, J., (1961) Ann.Intern.Med., 55, 33.

KAY, L.M. and TRUEBLOOD, K.M., (1949) Analyt.Chem., 21, 1566.

KERSHBAUM, A., BELLET, S., DICKSTEIN, E.R., and FEINBERG, L.J., (1961) Circulation Res., 9, 631.

KERSHBAUM, A., BELLET, S., CAPLAN, R.F., and FEINBERG, L.J. (1962) Am.J. Cardiol., 10, 204.

KERSHBAUM, A., and BELLET, S., (1964) J.Am.med.Ass., 187, 32.

KEULEMANS, A.I.M. (1959) "Gas Chromatography". New York: Reinhold Publishing Company.

KEYS, A., KIMURA, N., KUSUKAWA, A., BRONTE-STEWART, B., LARSEN, N., and KEYS, M.H., (1958) Ann.Intern.Med., 55, 33.

(1962)

KNOX, J.H., "Gas Chromatography". /London: Methuen & Co. Ltd., New York: John Wiley & Sons Inc. (ed. H.J. Emeleus, F.R.S., P.W.G. Style and R.P. Bell, F.R.S.)

KRUT, L.H., and BRONTE-STEWART, B., (1964) J.Lipid Res., 5, 343.

KURIEN, V.A., and OLIVER, M.F., (1966) Lancet, 2, 122.

LANDON, J., GREENWOOD, F.C., STAMP, C.B., and WYDA, V., (1966) J.clin.Invest., 45, 437.

LAUDAT, P., LEFEVRE, M., SALTIEL, H., and DE GENNES, J., (1966) J.Atheroscler.Res., 6, 192.

LAURELL, S., (1959) Acta physiol.scand., 46, 97.

LAURELL, S., (1960) Acta physiol.scand., 47, 218.

LAWRIE, S.G., McALPINE, S.G., PIRRIE, R., and RIFKIND, B.M., (1961) Clin.Sci., 20, 255.

LEBOEUF, B., FLINN, R.B., and CAHILL, G.F., (1959) Proc.Soc.exp. Biol.Med. 102, 527.

LEWIS, B., (1958) Lancet, 2, 71.

LINDGREEN, F.T., NICHOLS, A., and WILLS, R.D., (1961) Am.J.clin.Nutr., 9, 13.

LIPSKY, S.R., HAAVIK, A., HOPPER, C.L., and McDIVITT, R.W., (1957) J.clin.Invest., 36, 233.

LUDDY, F.E., BARFORD, R.A., and RIEMENSCHNEIDER, R.W., (1958) J.biol.Chem., 232, 843.

LYMAN, R.L., OSTWALD, R., BOUCHARD, P., and SHANNON, A., (1966) Biochem.J., 98, 438.

LYMAN, R.L., SHANNON, A., OSTWALD, R., and MILJANICH, P., (1964) Can.J. Biochem., 42, 365.

LYNN, W.S., MacLEOD, R.M., and BROWN, R.H., (1960) J.biol.Chem., 235, 1904.

MALINS, D.C., MANGOLD, H.K., (1960) J.Am.Oil Chemist Soc., 37, 576.

MATTSON, F.H., LUTTON, E.S. (1958) J. Biol. Chem. 233, 868

MEAD, J.F., and GOUZE, M.L., (1961) Proc.Soc.exp. Biol.Med., 106, 4.

MEINERTZ, H., (1962) Fedn.Proc.Fedn.Am.Socs.exp.Biol., 21, 284.

MEINERTZ, H., (1963) Fedn.Proc.Fedn.Am.Socs.exp.Biol., 22, 375.

MEURSING, E.H., (1962) Riv.Ital.Sost.Grasse, 4, 188 (quoted by Norcia & Evans, 1964).

MICHAELS, G.D., WHEELER, P., FUKAYAMA, G., and KINSELL, L.W., (1959) Ann.N.Y.Acad.Sci., 72, 633.

MILLER, H.I., GOLD, M., and SPITZER, J.J., (1962) Am.J.Physiol., 202, 370.

MOSES, C., and DANOWSKI, T.S., (1963) Metabolism, 12, 126.

MOSES, C., SUNDER, J.H. Vester, J.W., and DANOWSKI, T.S. (1964) Metabolism, 13, 717.

MOSINGER, F., (1965) J.Lipid Res., 6, 157.

MURCHISON, L.E., and FYFE, T., (1966), Lancet, 2, 182.

MYANT, N.B., and ILIFFE, J., (1963) in: Biochem.Soc. Symp. 24, "The Control of Lipid Metabolism", ed. by J.K. Grant. London: Acad. Press. pp.145-154.

NELSON, G.J., (1962) J.Lipid Res., 3, 71.

NELSON, W.R., WERTHESSEN, N.T., HOLLMAN, R.L., HAAWAY, H., and JAMES, A.T., (1961) Lancet, 1, 86.

NESTEL, J.P., (1964) J.clin.Invest., 43, 77.

NESTEL, J.P., (1965) Metabolism, 14, 1.

NESTEL, J.P., and COUZENS, E.A., (1966) J.clin.Invest., 45, 1234.

NESTEL, J.P., and STEINBERG, D., (1963) J.Lipid Res., 4, 461.

NEWMAN, H.A., McCANDLESS, E.I., and ZILVERSMIT, D.B., (1961) J.biol.Chem., 236, 1264.

NEWMAN, H.A., and ZILVERSMIT, D.B., (1962) J.biol.Chem., 237, 2078.

NICHANAN, M.Z., SWEELEY, C.C., OLDHAM, M.M., and OLSON, R.E., (1963) J.Lipid Res., 4, 484.

NORCIA, E.N., and EVANS, J.P., (1964) Metabolism, 13, 12.

NUTRITION REVIEWS (1965) 23, 238.

OKEY, R., LEE, M., HAMPTON, M.C., and MILIJANICH, P., (1960), Metabolism, 9, 791.

OLLEY, J., (1956) in: "Biochemical Problems of Lipids", Ed. Popjak, G., and Le Breton, E. London, Butterworth, p.49.

OLIVER, M.F., and BOYD, G.S., (1955), Symposium on Atherosclerosis, Minnesota Heart Association, p.64.

OLIVER, M.F., and BOYD, G.S., (1956) Lancet, 2, 1273.

OLIVER, M.F., and BOYD, G.S., (1958) Vitamins and Hormones, 16, 147.

OLIVER, M.F., and BOYD, G.S., (1959) in: "Hormones and Atherosclerosis" Ed. Pincus, G. New York: Academic Press, p.403.

ORTH, R.D., FINE, M.B., and WILLIAMS, R.H., (1961) Proc.Soc.exptl.Biol.Med., 106, 339.

PENICK, S.B., (1962) Clin.Res., 10, 402.

PHILLIPS, C., (1956) "Gas Chromatography". London: Butterworths.

PURNELL, H., (1962) "Gas Chromatography". London: John Wiley & Sons.

QUABBE, H.J., SCHILLING, E., and HELGE, H., (1966) J.clin.Endocr.Metab., 26, 1173.

QUARFORDT, S.H., and GOODMAN, De W.S., (1966) Biochim.biophys.Acta, 116, 382.

RABEN, M.S., and HOLLENBERG, C.H. (1959) Amer.J.Physiol., 39, 484.

RABEN, M.S., (1959) J.clin.Invest., 38, 1632 (Abstract)

RABINOWITZ, O., KLASSEN, G.A., and ZIEHLER, K.L., (1965) J.clin.Invest., 44, 51.

RANDLE, P.J., (1964) Post.grad.Med.J., 40, 457.

RAPER, H.S., (1907) J.Amer.chem.Soc., 91, 1831.

REAVEN, G.M., HILL, D.D., GROSS, R.C., and FARQUHAR, J.E., (1963) J.clin.Invest., 44, 1826.

RECANT, L., ALP, H., KOCH, M.B., and EGGEMAN, J., (1963) Lancet, 2, 614.

REITMAN, S., FRANKEL, S., (1957) Am.J.clin.Path., 28, 56.

RICH, C., BIERMAN, E.L., and SCWARTZ, I.L., (1959) J.clin.Invest., 38, 275.

RIFKIND, B.M., (1966) J.Atheroscler.Res., 6, 26.

ROBINSON, D.S., (1964) in "Metabolism and Physiological Significance of Lipids" ed. by R.M.C. Dawson and D.N. Rhodes, London-New York-Sidney, John Wiley and Sons. p.275-289.

ROHEIM, P.S., HAFT, D.E., GIDEZ, L.I., WHITE, A., and EDER, H.A., (1963) J.clin.Invest., 42, 1277.

ROTH, J., GLICK, S.M., YALOW, R.S. and BERSON, S.A. (1964) J.clin.Invest., 43, 1056.

ROTHLIN, M., and BING, R.J. (1961) J.clin.Invest., 40, 1380.

SAVARY, P., and DESNUELLE, P.D., (1954) Bull.Soc.Chim.France, 936. (quoted by Bottcher, 1959).

SCHRADE, W., BIEGLER, R., BÖHLE, E., and HARMUTH, E., (1963) Lancet, 1, 285.

SCHRADE, W., BÖHLE, E., BIEGLER, R., and SABEL, C., (1960) Klin.Wschr. 38, 707.

SCHRADE, W., BIEGLER, R., and BOHLE, E., (1961) J.Atheroscler.Res., 1, 47.

SCOT, R.F., LIKIMANI, J.C., MORRISON, E.S., THUKU, J.J., and THOMAS, W. (1963) Am.J.clin.Nutr., 13, 82.

SHAFRIR, E., and STEINBERG, D., (1960) J.clin.Invest., 39, 310.

SHAFRIR, E., SOSMAN, K.E., and STEINBERG, D. (1959) J.Lipid Res., 1, 109.

SINCLAIR, H.M., (1956) Lancet, 1, 381.

SMITH, A.F., (1967) Lancet, 1, 178.

SOLOFF, L.H., and SCHWARTZ, H., (1966) Lancet, 1, 449.

SPERRY, W.M., and WEBB, M., (1950) J.biol.Chem., 187, 97.

STARICH, R., and AMBANELLI, U., (1959) Gior.Clin.Med., 40, 2. (quoted by Richardson, J.A., Woods, E.F. and Bagwell, E.E. (1960) Am.J.Cardiol., 5, 613).

STEIN, Y., and SHAPIRO, B., (1959) Am.J.Physiol. 196, 1238.

STEIN, Y., and SHAPIRO, B., (1960) J.Lipid Res., 1, 326.

STEINBERG, D., (1963) in Biochem.Soc.Symp. 24 "The Control of Lipid Metabolism" ed. J.K. Grant, London: Academic Press.pp.111-143.

STOFFEL, W., CHU, W., and AHRENS, E.H. (1959) Analyt.Chem., 31, 307.

SWELL, L., FIELD, H., and TREADWELL, G.R., (1960) Proc.Soc.exp.Biol.Med., 105, 129.

SWELL, L., SCHOOLS, P.E., and TREADWELL, G.R. (1962) Proc.Soc.exp.Biol.Med., 109, 682.

TRAPPE, W., (1940) Biochem.J., 305, 150.

TROUT, D.L., ESTES, E.H., and FREIDBERG, S.J., (1960) J.Lipid Res., 1, 199.

TRUEBLOOD, K.N., and MALMBERG, E.W., (1954) Analyt.Chem., 26, 1055.

TUNA, N., RECKERS, L., and FRANTZ, J.D. (1958) J.clin.Invest., 37, 1158.

UZAWA, H., MICHAELS, G., COELHO, M., KINSELL, L., (1964) Clin.Res., 12, 119.

VAUGHAN, M., and STEINBERG, D., (1963) J.Lipid Res., 4, 193.

VAUGHAN, M., and STEINBERG, D., (1962) Fedn.Proc.Fedn.Am.Socs.exp.Biol., 21, 284.

WAGNER, O., (1926) Biochem.Z., 174, 412.

WALLACE, L.C., and BASSET, J.M., (1966) Metabolism, 15, 95.

WERTHEIMER, E., and SHAFRIR, E., (1960) Recent Prog.Horm.Res., 16, 467.

WHYTE, H.M., GOODMAN, De W.S., and KARMEN, A., (1963) in "Biochemical Problems of Lipids" Ed.: A.C. Frazer. Elsevier Publishing Company, Amsterdam-London-New York. pp.229-236.

WILSON, W.R., and HANSEN, A.E., (1935) J.biol.Chem., 112, 457.

WORLD HEALTH ORGANISATION, (1959) Techn.Rep.Ser.Wld.Hlth.Org. No. 168.

WRIGHT, A.S., PITT, G.A.J., and MORTON, R.A., (1959) Lancet, 2, 594.

ZILVERSMIT, D.B., and McCANDLESS, E.L., (1959) J.Lipid Res., 1, 118.

ZILVERSMIT, D.B., SWELLEY, C.C., and NEWMAN, H.A., (1961) Circulation Res., 9, 235.

ACKNOWLEDGEMENTS

I should like to thank Dr. M.F. Oliver for providing facilities for this study, for his helpful suggestions and constant encouragement.

I wish to express my warmest gratitude to Professor L.G. Whitby for the hospitality of his Department and for his criticism which was always constructive.

The clinical material for this study was provided by Dr. M.F. Oliver. The clinical cooperation of Drs. M. Sznajderman, and V.A. Kurien in the study of human growth hormone and Dr. M. Godman in the study of noradrenaline is gratefully acknowledged.

I wish to thank Mrs. S. Conrad for her skilled technical assistance, Mr. T. Healy for mechanical care for the gas chromatographic equipment, and Miss J. Yule for help in routine analyses.

Reprinted from
Clinica Chimica Acta
Elsevier Publishing Company,
Amsterdam
Printed in The Netherlands

THE ESTIMATION OF SERUM CHOLESTEROL

JADWIGA JURAND AND F. ALBERT-RECHT
Department of Clinical Chemistry, University of Edinburgh (Great Britain)

(Received January 27th, 1962)

Many colour reactions for the determination of cholesterol have been described. Since a number of serum constituents, such as proteins, pigments etc. form coloured complexes with the reagents employed, methods in which cholesterol is isolated before estimation are the more desirable[1, 2]. The classic Liebermann-Burchard reaction has several disadvantages, as the reaction is sensitive to light, dependent on temperature, and the resulting colour changes from minute to minute[3, 4]. ZLATKIS *et al.*[5] used ferric chloride in glacial acetic acid and concentrated sulphuric acid. TRINDER[6] has found that different organic halides mixed with sulphuric acid give reddish colours with cholesterol and devised a method based upon the reaction between a mixture of acetyl chloride, concentrated sulphuric acid and solution of cholesterol in ethylene dichloride. Recently SEARCY AND BERGQUIST[7] described a colorimetric method for the estimation of serum cholesterol based on the observation that a saturated solution of ferrous sulphate in glacial acetic acid and concentrated sulphuric acid develops an orange colour if added to an acetone–alcohol solution of cholesterol.

This paper describes a method for the quantitation of total serum cholesterol using the extraction technique of ABELL *et al.*[8] as modified by ANDERSON AND KEYS[9] and a colour development based on the reaction between an alcoholic solution of cholesterol and a mixture of glacial acetic acid and concentrated sulphuric acid.

MATERIALS AND METHOD

Reagents

(1) Absolute alcohol, redistilled. (2) Glacial acetic acid, redistilled. (3) Concentrated sulphuric acid, analytical reagent grade (B.D.H.). (4) Potassium hydroxide solution 33% (w/w), *i.e.* 10 g of potassium hydroxide analytical reagent grade dissolved in 20 ml of water. (5) Alcoholic potassium hydroxide solution made immediately before use by adding 6 ml of 33% KOH to 94 ml of absolute alcohol. (6) Petroleum ether b.p. 40–60°, redistilled. (7) Standard cholesterol solution containing 120 mg cholesterol (recrystallised once from glacial acetic acid, three times from absolute alcohol and dried to constant weight) in 100 ml of absolute alcohol. This standard, containing 480 μg/0.4 ml, is diluted with absolute alcohol to give 120 μg and 240 μg/0.4 ml. (8) Colour reagent: 1 vol. of concentrated sulphuric acid is added to 4 vols. of redistilled glacial acetic acid. The mixture is shaken and used after reaching room temperature. The reagent can be kept for 2–3 days.

Procedure

Into a 15-ml glass-stoppered tube containing 2 ml of freshly prepared alcoholic potassium hydroxide 0.10 ml of serum is carefully pipetted. After incubation at

37–40° for 90 min and addition of 2 ml of water, the mixture is extracted twice with 4 ml of freshly redistilled petroleum ether by shaking for 60 sec. Centrifugation for 3 min at 1500 rev./min helps separation of phases. The organic phase is transferred by Pasteur pipette into another dry glass-stoppered tube and the pipette is rinsed with a little petroleum ether, the washings being added to the organic phase. (The above extraction technique is identical with that used by ANDERSON AND KEYS[9]). The petroleum ether is evaporated at 60° in a vacuum with an air leak. The residue is placed in an air oven at 75 ± 1° for 3 min, removed and when slightly warm 0.4 ml of absolute alcohol is added to each tube. With each batch of determinations a blank with 0.4 ml of absolute alcohol and a series of 0.4 ml of alcoholic solutions containing 120, 240 and 480 μg of cholesterol are prepared in duplicate. 5 ml of the acetic sulphuric mixture is added to each tube. The tubes are stoppered and are immediately mixed by gently inverting a few times. The stoppered tubes are now incubated for 20 min at 75 ± 1°. After cooling the tubes to room temperature, the optical density is read at 500 mμ. The colour is stable for 3–4 h after incubation. The results are read off a graph constructed by plotting optical densities against cholesterol concentration of standards.

RESULTS AND DISCUSSION

The absorption spectrum

The characteristic absorption spectrum obtained when using the finally adopted method shows a minor peak in the region of 385–415 mμ, shallow at low concentrations of cholesterol, and a main peak at 500 mμ.

The effect of acetic acid and sulphuric acid proportions on the optical densities of standard solutions

Standards containing 120, 240, 480 μg of cholesterol at 0.4 ml of absolute alcohol were mixed with 5 ml of colour reagent containing acetic acid and sulphuric acid in varying proportions. The absorption spectra show that with an increase in the proportion of acetic acid, there is a decrease in the optical density in the 385–415 region and a shift of the absorption maximum from 410 to 385 mμ associated with an increase in the extinction at 500 mμ, the colour being changed from yellow to red.

Curves of optical density at 500 mμ plotted against concentration show that the optimal linearity is achieved with 4 : 1 (v/v) acetic to sulphuric acid, Beer's law being obeyed within the range of concentration used.

Influence of the nature of the solvent employed

If dried cholesterol is treated with the colour reagent alone, the resulting mixture shows varying degrees of turbidity. After centrifugation, however, the clear supernatant shows absorption spectrum maxima at 500 mμ and 395 mμ. Formation of the orange-red colour occurs in the absence of any solvent, but to obtain a turbidity-free solution such as is necessary for spectrophotometry requires use of a solvent prior to addition of colour reagent.

When cholesterol standards were dissolved in 0.4, 0.7, 1.0 ml of ethanol it was found that at 500 mμ optimum linearity of the optical density/concentration curve was obtained when 0.4 ml of ethanol was used.

When, however, acetone–alcohol (1 : 1) was used as the solvent for the standards, the curve of optical density at 500 mμ against concentration showed no such linearity.

Effect of temperature and mixing procedure on colour development

If a standard cholesterol solution is mixed with acetic acid followed by sulphuric acid, the mixing is accompanied by evolution of heat and the colour development is complete in about 15 min. If the colour reagent is mixed prior to adding it to the standard, the colour develops slowly, but on incubation at 75° for 20 min the colour develops fully and remains constant at room temperature for about 4 h. The character of the absorption spectrum is the same whichever technique is used, and the ultimate optical density is very similar.

Optimal reproducibility was obtained by allowing the colour reagent to run down the side of the test tube and mixing it with the standard solution by gentle shaking.

Effect of ferric and ferrous salts on the colour development

Other authors have included ferric[5] or ferrous[7] salts in the colour reagent to enhance the development of colour. While investigating the present method, analytical grade reagents were used for preparation of colour reagent, without addition of either ferric or ferrous salts. Some characteristics of the absorption spectra and its changes under various conditions suggested that the presence of catalytic quantities of iron ordinarily present as impurity (up to 100 μg/100 ml) even in the analytical grade of sulphuric acid may be required for the colour development.

Ferric salts: The influence of ferric iron in the colour formation was tested by using very dilute solutions of varying concentration of ferric ions (added as chloride) in redistilled glacial acetic acid. These solutions were used in the preparation of the colour reagent. Then 0.4-ml portions of alcoholic solutions of cholesterol containing 120, 240 and 480 μg of cholesterol were mixed with the colour reagent, incubated and the spectra of all samples were noted. Fig. 1 shows the results with 120 μg of cholesterol. The optical densities of standards at 500 mμ increase slightly with increasing concentration of Fe^{3+} in the colour reagent. However, there is a critical concentration in relation to the concentration of cholesterol. When this is exceeded, there is a decrease in optical density at 500 mμ and the character of the absorption spectrum changes. The curve relating optical density at 500 mμ to cholesterol concentration is non-linear at the higher concentrations of Fe^{3+}.

Ferrous sulphate: If colour is developed with a reagent incorporating acetic acid that has been saturated with ferrous sulphate, different results are obtained by using varying amounts of ferrous sulphate for saturation (Fig. 2). When 0.2, 0.5 and 1.0 g of ferrous sulphate are used for saturation of 100 ml of glacial acetic acid the absorption spectra show a close similarity to those obtained with low concentrations of ferric chloride.

In all cases the optical density at 500 mμ was greater than in the absence of added ferrous sulphate; there appeared to be an optimum in this respect amount since the optical density was actually less when 1 g $FeSO_4$ was used than when only 0.5 or 0.2 g was used for saturation. However, the range linearity of the response was greatest in the absence of added ferrous sulphate.

If ferrous sulphate were the substance which is responsible for colour develop-

ment, the character and degree of colour should be the same regardless of the quantity of ferrous sulphate used for saturation. It is, therefore, likely that the presence of other substances than ferrous sulphate, probably ferric impurities, is mainly responsible for the increase of the extinction at 500 mμ. This point was proved by the following experiment.

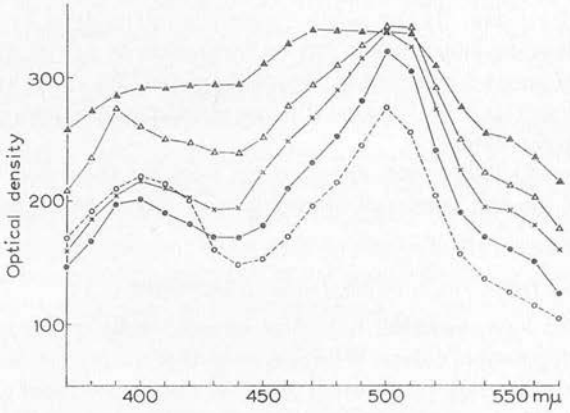


Fig. 1. Absorption spectra obtained by varying the concentration of ferric chloride in the glacial acetic acid in the reaction with 120 μg of cholesterol. The colour reagent with pure acetic acid (○---○), with acetic acid containing 0.5 μg Fe^{3+}/ml (●—●), 1 μg Fe^{3+}/ml (×—×), 2 μg Fe^{3+}/ml (△—△), 5 μg Fe^{3+}/ml (▲—▲).

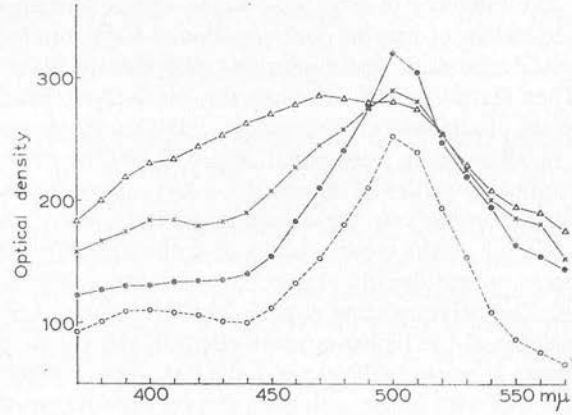


Fig. 2. Effect of saturation over different amounts of ferrous sulphate. Spectra of 120 μg of cholesterol with pure acetic acid (○---○), with acetic acid saturated with ferrous sulphate using 0.2 g/100 ml (●—●), 0.5 g/100 ml (×—×) and 1 g/100 ml (△—△).

One and the same sample (0.2 g) of ferrous sulphate was used consecutively for saturation of three separate 50-ml aliquots of acetic acid. The saturation was accomplished by shaking the mixture for 15 min under nitrogen to avoid presence of air. After centrifugation the three supernatant aliquots were used for preparation of the colour reagent. The spectra showed that the optical density at 500 mμ using the first aliquot was higher than that using the second aliquot (Fig. 3). This is probably due to a higher concentration of ferric or other impurities in the aliquot used for the

first "washing" than for the second. The more soluble ferric salts, which are present as impurities in the ferrous sulphate salt were probably largely removed with the first portion of acetic acid. The third aliquot showed a slightly lower extinction at 500 mμ, the diminution being less than that between the first and second, but the extinction was still higher than with a colour reagent to which no ferrous sulphate had been added. This can be explained by supposing that the active impurity re-forms every time during the procedure of saturation, or that the ferrous iron present in the solution of glacial acetic acid in traces (ferrous sulphate is practically insoluble in glacial acetic acid) is oxidised during the reaction with acetic and sulphuric acid.

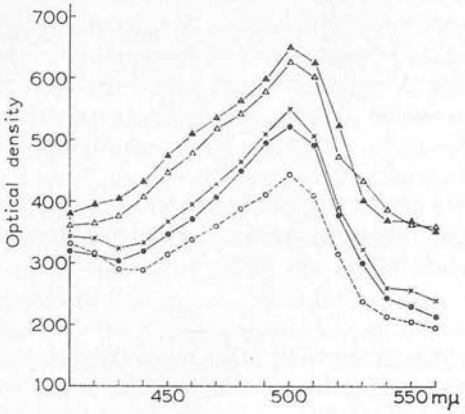


Fig. 3. Consecutive saturation of three 50-ml aliquots of glacial acetic acid with the same 0.2 g $FeSO_4$ and the effect of aeration. Spectra of 240 μg of cholesterol. The colour reagent contains pure acetic acid (○---○); acetic acid used as first aliquot (first "washing") (△—△); as second "washing" (×—×); as third "washing" (●—●); as "washing" of the aerated sample (▲—▲).

To show how the presence of oxygen changes the property of ferrous sulphate used for the preparation of the colour reagent, 0.2 g of ferrous sulphate was washed with 50 ml of glacial acetic acid, centrifuged and the supernatant used as the "first washing". A stream of oxygen was then blown into the washed ferrous sulphate for 2 h. A fresh 50-ml aliquot of acetic acid was saturated with this ferrous sulphate and used for preparation of a colour reagent. This gave colour showing optical density slightly higher than the first washing (Fig. 3). This would also suggest that the increase of the colour reaction is due to presence of iron in the ferric state.

Evaluation of the finally adopted method

Reproducibility: On determining 10 times the cholesterol content of 3 separate serum samples the results were as follows:

TABLE I

	Mean (mg/100 ml)	*Standard deviation*	*Coefficient of variation*
Serum I	211	± 2.9	1.42
Serum II	239	± 4.25	1.68
Serum III	332	± 4.65	1.5

Recoveries: Varying amounts of cholesterol standard solution in alcohol were added to 2 ml of alcoholic potassium hydroxide solution prior to adding 0.1 ml of serum, and the percentage recovery was determined. The results are given in Table II.

TABLE II

RECOVERY OF CHOLESTEROL ADDED TO SERUM

Cholesterol added (μg)	*Cholesterol found (μg)*	*Cholesterol recovered* (μg)	(Percentage)
0	305	—	—
120	428	123	102.5
180	485	180	100
240	537	232	97

Normal range

52 normal sera were analysed with the present method. The average cholesterol concentration was 183 mg/100 ml of serum. The normal range calculated on the basis of ± 2 S.D. was found to be 115–251 mg%.

Comparison of results

92 blood samples from normal and atherosclerotic patients were analysed using the ANDERSON AND KEYS extraction technique. The colour was developed by the Liebermann-Burchard reaction and also by the present procedure. 50 of the same samples were analysed by the method of SPERRY AND WEBB[10]. The results can be seen in Fig. 4a and b. No significant differences have been found between the results given by the two other methods and the method here described.

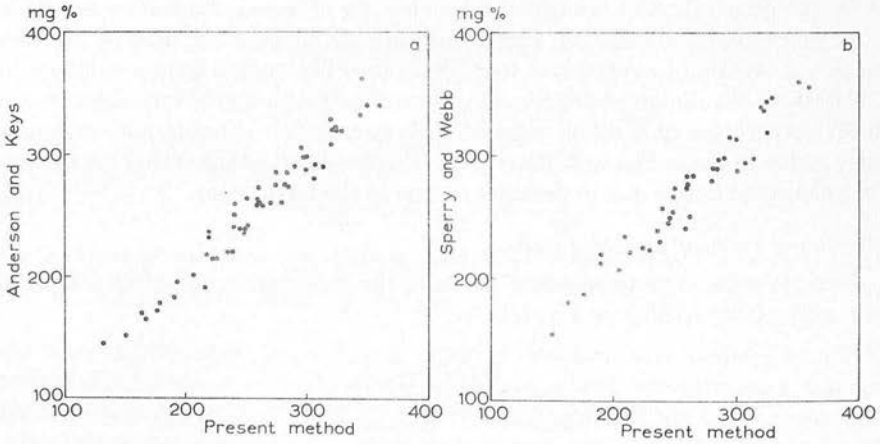


Fig. 4. (a) Comparison between the results obtained by the method of ANDERSON AND KEYS and the present method. (b) Comparison between the results obtained by the method of SPERRY AND WEBB and the present method.

ACKNOWLEDGEMENTS

We should like to thank Dr. C. P. STEWART for his support and interest in this work and Dr. M. F. OLIVER for making it possible for this work to be undertaken. This study has been supported by a grant from the Scottish Hospital Endowments Research Trust, and one of us (J.J.) acknowledges receipt of a personal grant from Glaxo Laboratories Ltd.

SUMMARY

A colorimetric method for serum cholesterol estimation has been described. The extraction method of ABELL *et al.*, as modified by ANDERSON AND KEYS, was adopted and the extracted cholesterol was determined by the colour reaction with mixture of acetic and sulphuric acids. The advantages of the method are: (1) The stability of the optical density over a period of hours, (2) the stability of the reagent for 2 or 3 days, (3) the high degree of reproducibility of the results, and (4) the concordance with the method of ANDERSON AND KEYS using the colour reagent of Liebermann-Burchard and with that of SPERRY AND WEBB.

A colour reaction obtained by the addition of colour reagent containing acetic acid and sulphuric acid to cholesterol and to cholesterol solutions has been studied, and the effect of ferric and ferrous iron on the colour formation was investigated. It was concluded that Fe^{3+} in trace amounts was responsible for the efficacy of the colour reagent and that B.D.H. sulphuric acid (Analytical Grade) contained a suitable concentration.

REFERENCES

1 R. G. HERMANN, *Proc. Soc. Exptl. Biol. Med.*, 94 (1957) 503.
2 T. G. MORRIS, *J. Clin. Pathol.*, 12 (1959) 518.
3 W. M. SPERRY AND F. C. BRAND, *J. Biol. Chem.*, 150 (1943) 315.
4 A. SAIFER AND O. F. KAMMERER, *J. Biol. Chem.*, 164 (1946) 657.
5 A. ZLATKIS, B. ZAK AND A. J. BOYLE, *J. Lab. Clin. Med.*, 41 (1953) 486.
6 P. TRINDER, *Analyst*, 77 (1952) 321.
7 R. L. SEARCY AND L. M. BERGQUIST, *Clin. Chim. Acta*, 5 (1960) 192.
8 L. L. ABELL, B. B. BRODIE AND F. E. KENDALL, *J. Biol. Chem.*, 195 (1952) 357.
9 J. T. ANDERSON AND A. KEYS, *Clin. Chem.*, 2 (1956) 145.
10 W. M. SPERRY AND M. WEBB, *J. Biol. Chem.*, 187 (1950) 97.

Reprinted from
Journal of Atherosclerosis Research
Elseviers Publishing Company
Amsterdam
Printed in the Netherlands

THE EFFECTS OF ETHYL CHLOROPHENOXYISOBUTYRATE ON SERUM CHOLESTERYL, TRIGLYCERIDE AND PHOSPHOLIPID FATTY ACIDS

JADWIGA JURAND AND M. F. OLIVER

Departments of Clinical Chemistry and Cardiology, Royal Infirmary, Edinburgh (Scotland)

INTRODUCTION

Ethyl-α-p-chlorophenoxyisobutyrate (CPIB) has been shown to lower serum cholesterol and serum triglycerides[1], to reduce the S_f 0–20 group of low-density lipoproteins[2] and serum free fatty acids[3] and also, insofar as it is almost certainly the active component of Atromid (CPIB in combination with androsterone), to decrease the total exchangeable pool of lipoprotein and the catabolic rate of the protein[4].

This paper reports a small study of the effects of CPIB on the composition of certain serum esterified fatty acids.

DESIGN OF STUDY

Fasting blood samples were obtained from 20 men with ischaemic heart disease. The criteria for their selection have been outlined earlier in this symposium[5]. These men were placed in four groups and aliquots of serum from each man were pooled accordpreing to the groups indicated:

Group A: 6 men without treatment;
Group B: 3 men on CPIB 2 g daily for 8–14 weeks;
Group C: 3 men on CPIB 2 g daily for 8–14 weeks;
Group D: 6 men without treatment, but CPIB had been given until 4–6 weeks previously.

In the two remaining men, the effect of CPIB 2 g daily was studied sequentially. One of these (I.K.) had a fatty acid spectrum observed in other men with ischaemic heart disease; the second man (R.Z.) had abnormally high concentrations of eicosa- and docosa-unsaturated acids. The individual controls were taken one week and one day before treatment.

METHODS

A total lipid extract was prepared from serum by the method of FOLCH *et al.*[6].

The serum cholesterol was estimated by the method of JURAND AND ALBERT-RECHT[7]. Serum triglycerides were estimated by CARLSON's modified method[8]. The lipids were separated by column chromatography using hydrated silicic acid for the

separation of phospholipids and hydrated florisil for the separation of cholesteryl and glyceryl esters. Intermethylation of these lipid fractions was undertaken according to the procedure of STOFFEL *et al.*[9]. The fatty acid composition of these methyl esters was then analysed by gas–liquid chromatography (using a Pye Argon chromatograph). Full details of the analytical procedure adopted in this laboratory will be published separately.

TABLE I

FATTY ACID COMPOSITION OF CHOLESTERYL ESTERS IN MEN WITH ISCHAEMIC HEART DISEASE WITHOUT TREATMENT AND RECEIVING CPIB

Fatty acids (short-hand designation)	*Pooled serum from 6 men without treatment*	*Pooled serum from 2 groups of 3 men receiving CPIB*		*Pooled serum from 6 men one month after CPIB was stopped*
		Group 1	*Group 2*	
	TOTAL CHOLESTEROL (mg/100 ml)			
	287	250	267	310
	FATTY ACIDS (% of total)			
14 : 0	0.27	0.94	0.47	0.63
16 : 0	8.32	9.40	9.20	8.60
16 : 1	5.36	7.10	6.28	4.30
18 : 0	0.14	0.90	1.03	0.37
18 : 1	*18.50*	*27.10*	*25.50*	*18.90*
18 : 2	*56.00*	*41.60*	*46.00*	*57.70*
*	tr	1.50	1.15	tr
18 : 3	0.33	0.63	0.17	0.45
20 : 3	0.59	0.59	0.34	0.77
20 : 4	8.90	9.20	9.60	8.43
20 : 5 + 22 : 1	1.70	1.20	0.95	1.44
Ratio 18 : 2/18 : 1	3.10	1.57	1.73	3.05

* Unknown. ECL on polyester = 19,20.

RESULTS

The principle changes in fatty acid composition derived from pooled serum samples are shown in Tables I–III. The similarity of the composition of fatty acids between Groups B and C (CPIB-treated), on the one hand, and Groups A and D (untreated) on the other, is worth emphasising. The changes in fatty acid composition derived from individual studies are shown in Tables IV–V.

Cholesteryl esters

(1) *Pooled serum samples* (Table I). The main changes in cholesteryl esters are an increase in the percentage composition of oleate, to a lesser extent of palmitoleate and also perhaps palmitate, and a decrease in linoleate.

TABLE II

FATTY ACID COMPOSITION OF GLYCERYL ESTERS IN MEN WITH ISCHAEMIC HEART DISEASE WITHOUT TREATMENT AND RECEIVING CPIB

Fatty acids (short-hand designation)	*Pooled serum from 6 men without treatment*	*Pooled serum from 2 groups of 3 men receiving CPIB*		*Pooled serum from 6 men one month after CPIB was stopped*
		Group 1	*Group 2*	
	TRIGLYCERIDES (mmoles/l)			
	1.68	1.61	1.79	1.88
	FATTY ACIDS (% of total)			
14 : 0	2.08	2.10	1.87	3.10
16 : 0	25.40	24.10	24.30	26.00
16 : 1	6.53	8.20	7.32	6.90
18 : 0	4.27	3.19	2.48	3.60
18 : 1	*42.50*	*43.00*	*45.80*	*39.00*
18 : 2	*17.10*	*13.50*	*13.30*	*16.50*
*	tr	0.50	0.82	0.49
18 : 3	0.27	0.44	0.20	0.36
20 : 0	0.26	0.30	0.20	0.12
20 : ?	0.45	0.40	0.14	0.52
20 : 2	0.20	0.10	0.10	0.20
20 : 2	0.10	0.26	0.32	0.10
20 : 3	0.20	0.10	0.15	0.30
20 : 4	1.10	3.24	3.10	2.01
20 : 5 + 22 : 1	0.52	0.60	0.60	0.50
Ratio 18 : 2/18 : 1	0.40	0.31	0.28	0.42

* Unknown. ECL on polyester = 19,20.

TABLE III

FATTY ACID COMPOSITION OF PHOSPHOLIPID ESTERS IN MEN WITH ISCHAEMIC HEART DISEASE WITHOUT TREATMENT AND RECEIVING CPIB

Fatty acids (short-hand designation)	*Pooled serum from 6 men without treatment*	*Pooled serum from 2 groups of 3 men receiving CPIB*		*Pooled serum from 6 men one month after CPIB was stopped*
		Group 1	*Group 2*	
14 : 0	0.15	0.06	0.17	0.06
16 : 0	25.60	24.10	24.70	28.80
16 : 1	0.31	1.01	1.39	0.90
16 : 3? 18 : br	0.30	0.57	0.47	0.28
18 : 0	13.40	12.60	12.50	13.30
18 : 1	*13.90*	*16.00*	*16.70*	*12.30*
18 : 2	*20.20*	*17.30*	*19.80*	*22.10*
18 : 3	0.11	0.18	0.19	0.12
20 : 0	0.45	0.48	0.29	0.33
20 : 2	0.50	1.39	0.83	0.44
20 : 3	3.70	4.02	3.47	3.10
20 : 4	11.00	12.90	12.60	9.50
20 : 5 + 22 : 1	2.98	3.52	1.57	2.72
22 : 5	1.82	1.85	1.54	1.66
22 : 6	5.20	3.70	3.02	3.88
Ratio 18 : 2/18 : 1	1.51	1.08	1.17	1.80

TABLE IV

THE EFFECT OF CPIB TREATMENT ON THE FATTY ACID COMPOSITION OF CHOLESTERYL, GLYCERYL AND PHOSPHOLIPID ESTERS IN A MAN (I.K.) WITH ISCHAEMIC HEART DISEASE

Fatty acids (short-hand designation)	*Cholesteryl fatty acids*				*Glyceryl fatty acids*				*Phospholipid fatty acids*			
	1st control	*2nd control*	*2 weeks on CPIB*	*4 weeks on CPIB*	*1st control*	*2nd control*	*2 weeks on CPIB*	*4 weeks on CPIB*	*1st control*	*2nd control*	*2 weeks on CPIB*	*4 weeks on CPIB*
	TOTAL CHOLESTEROL (mg/100 ml)				TOTAL TRIGLYCERIDES (mmoles/l)							
	289	334	231	207	1.35	1.39	0.60	1.12	–	–	–	–
	FATTY ACIDS (% of total)											
14 : 0	0.45	0.35	0.38	0.60	1.41	0.80	0.70	0.85	0.25	0.44	0.26	0.25
16 : 0	8.04	8.70	7.90	8.10	22.70	22.00	26.70	21.00	24.60	23.30	24.90	24.20
16 : 1	*2.10*	*2.90*	*3.50*	*4.60*	3.51	3.24	4.48	4.00	0.75	0.78	1.10	1.20
16 : 3? 18 : br									0.36	0.47	0.35	0.37
18 : 0	0.56	0.31	0.99	0.63	4.52	3.99	2.90	2.52	*13.70*	*14.50*	*11.10*	*19.80*
18 : 1	*18.80*	*18.55*	*24.70*	*24.30*	46.90	46.30	46.50	48.80	*10.70*	*11.10*	*16.60*	*17.00*
18 : 2	*57.3*	*57.4*	*50.00*	*49.30*	16.35	19.80	14.80	14.65	*22.60*	*23.30*	*20.60*	*20.60*
18 : ?*			1.1	1.86								
18 : 3	0.22	0.17	0.35	0.29	0.95	0.75	0.52	0.48	0.18	0.10	0.26	0.72
20 : 0					0.10	0.10	0.30	0.96	0.50	0.59	tr	tr
20 : 1 + 20 : ?					0.50	0.20	0.20	0.86				
20 : 2					0.17	0.12	0.20	0.20	0.22	0.10	0.56	0.70
20 : 3	0.10	tr	0.30	tr	0.10	0.20	tr	0.10	1.47	1.50	2.86	3.12
20 : 4	11.8	10.1	9.82	9.30	*2.20*	*2.60*	*2.90*	*4.63*	13.40	13.90	11.90	12.90
20 : 5 + 22 : 1	0.60	1.60	1.65	1.10	0.63	0.20	0.25	1.20	2.88	2.89	2.25	2.02
22 : 5 + 24 : 1									1.60	1.85	1.27	1.67
22 : 6 + 24 : ?									6.40	5.85	6.03	6.45
Ratio 18 : 2/18 : 1	3.04	3.09	2.06	2.05	0.35	0.42	0.31	0.30	2.1	1.99	1.28	1.21

* ECL on polyester = 19,20.

TABLE V

THE EFFECT OF CPIB TREATMENT ON THE FATTY ACID COMPOSITION OF CHOLESTERYL, GLYCERYL AND PHOSPHOLIPID ESTERS IN A MAN (R.Z.) WITH ISCHAEMIC HEART DISEASE

Fatty acids (short-hand designation)	*Cholesteryl fatty acids*				*Glyceryl fatty acids*				*Phospholipid fatty acids*			
	1st control	*2nd control*	*2 weeks on CPIB*	*4 weeks on CPIB*	*1st control*	*2nd control*	*2 weeks on CPIB*	*4 weeks on CPIB*	*1st control*	*2nd control*	*2 weeks on CPIB*	*4 weeks on CPIB*
	TOTAL CHOLESTEROL (mg/100 ml)				TOTAL TRIGLYCERIDES (mmoles/l)							
	278	296	199	196	3.24	2.96	1.25	1.80	–	–	–	–
					FATTY ACIDS (% of total)							
12 : 0					0.96	0.65	tr	tr				
14 : 0	0.50	0.79	0.81	0.55	4.10	3.66	0.84	1.79	0.20	0.20	tr	0.29
16 : 0	7.90	7.35	9.14	8.00	24.80	23.30	22.70	26.00	23.00	20.60	17.00	23.40
16 : 1	*4.10*	*5.10*	*6.10*	*6.50*	4.90	5.70	5.17	6.85	*0.24*	*0.10*	*3.36*	*1.49*
16 : 3									0.19	0.55	tr	0.46
18 : 0	0.11	0.10	0.27	0.45	5.25	4.89	3.08	2.91	*11.31*	*13.80*	*7.80*	*9.90*
18 : 1	*18.00*	*19.00*	*23.05*	*25.40*	*39.90*	*38.60*	*42.30*	*42.60*	*12.90*	*10.70*	*14.70*	*16.10*
18 : 2	*50.60*	*52.70*	*42.10*	*36.40*	*14.30*	*14.35*	*12.70*	*9.40*	*18.10*	*14.20*	*13.80*	*13.90*
18 : ?*	tr	0.30	2.26	2.60	tr	tr	0.93	0.76				
18 : 3	tr	tr	1.65	1.50	1.51	1.80	0.82	1.20				
20 : 0												
20 : 1 + 20 : ?					0.74	1.16	tr	1.32	tr	0.58	0.37	0.75
20 : 2					0.67	0.59	0.70	0.50	0.81	1.00	0.29	tr
20 : 3	0.67	0.40	0.20	3.08	tr	tr	tr	tr	2.18	2.05	2.06	2.47
20 : 4	10.25	7.64	8.40	11.10	*1.60*	*2.26*	*4.66*	*3.52*	10.90	10.95	10.75	6.30
20 : ?												2.98
20 : 5 + 22 : 1	7.95	6.34	6.30	4.43	1.38	2.59	6.26	3.50	5.00	9.10	9.50	4.26
22 : 4									tr	tr	1.62	1.91
22 : ?**											1.78	3.56
22 : 5									3.67	1.67	2.98	3.38
22 : 6 + 24 : ?									11.30	14.90	12.15	8.00
Ratio												
18 : 2/18 : 1	2.80	2.76	1.82	1.44	0.36	0.37	0.30	0.22	1.40	1.32	0.94	0.86

* ECL on polyester = 19,20; ** ECL on polyester = 21,60.

(2) *Individual studies* (Tables IV and V). The results of the sequential individual studies show the same changes as those observed from pooled serum.

Triglyceride esters

(1) *Pooled serum samples* (Table II). Although the results show a small increase in the percentage composition of oleate, palmitoleate and arachidonate with a small decrease in stearate and linoleate, it is difficult to conclude that these changes are solely due to the effect of CPIB because the usual spectrum of fatty acid composition of triglycerides shows a wide range of variability.

(2) *Individual studies* (Tables IV and V). These results confirm that there is a small rise in oleate and arachidonate with a small decrease in stearate and linoleate.

Phospholipid esters

(1) *Pooled serum samples* (Table III). The main changes produced in phospholipid esters are an increase in oleate and palmitoleate and a decrease in stearate and in linoleate.

(2) *Individual studies* (Tables IV and V). These results confirm the trends observed from pooled serum.

DISCUSSION

These results should be regarded as preliminary in nature. There are obvious fallacies in studying groups of pooled serum from different men with ischaemic heart disease with a greater or lesser degree of atherosclerosis. Yet, most of the trends observed from pooled serum after CPIB treatment are confirmed by the individual studies.

ACKNOWLEDGEMENT

We should like to thank Professor L. G. WHITBY for his criticism and Mrs. S. CONRAD and Mr. T. HEALY for their expert technical assistance. This research has been partly financed by a grant from the Scottish Hospitals Endowments Research Trust.

SUMMARY

Gas chromatographic analysis has been made of the esterified fatty acid composition of pooled sera obtained from men with ischaemic heart disease before, during and after the administration of CPIB, and of sera obtained from two men followed sequentially before and during CPIB treatment. The main changes were observed in serum cholesteryl and phospholipid esters. In both fractions, CPIB increased the oleate and palmitoleate content and decreased linoleate; phospholipid stearate was also decreased. The changes observed in triglycerides were less striking although the trends were the same as for cholesteryl and phospholipid esters and, in addition, glyceryl arachidonate increased after CPIB treatment.

RÉSUMÉ

Il a été fait une analyse par chromatographie gazeuse de la composition en acides gras estérifiés de sérum recueilli chez des hommes avec cardiopathie ischémique, avant, pendant et après administration de CPIB ainsi que de sérum recueilli chez deux hommes suivis continuellement avant et pendant le traitement par le CPIB. On a noté les changements les plus importants dans le cholestéryle sérique et les esters de phospholipides. Dans les deux fractions, le CPIB a augmenté la teneur en oléate et en palmitoléate et diminué celle en linoléate; le stéarate phospholipidique a diminué aussi. Les changements observés dans les triglycérides étaient moins accusés bien que les tendances fussent les mêmes que pour les esters de cholestéryle et de phospholipides et que, de plus, l'arachidonate de glycéryle ait augmenté après traitement par le CPIB.

ZUSAMMENFASSUNG

Eine chromatographische Gasanalyse wurde gemacht von dem veresterten Fettsäuregemisch gepoolter Seren von Patienten mit ischämischem Herzleiden, vor während und nach der Verabreichung von CPIB; ebenfalls wurden Seren zweier Männer, die serienweise vor und während CPIB-Behandlung überprüft wurden, gaschromatographisch untersucht. Die Hauptveränderungen wurden im Serumcholesterin und in den Esterphospholipoiden beobachtet. Bei beiden Fraktionen erhöhte CPIB den Oleinat- und Palmito-Oleinat-Gehalt und verringerte das Linoleat. Auch das Phospholipoid-Stereat war vermindert. Die Veränderungen der Triglyzeriden war weniger eindrucksvoll, obzwar die Tendenz dieselbe war wie die für Cholesterin und die Esterphospholipoiden, und auch das Glyzerin-Arachidonat nach CPIB-Behandlung zunahm.

REFERENCES

1 M. F. OLIVER, *Lancet*, i (1962) 1321.
2 E. H. STRISOWER, *J. Atheroscler. Research*, 3 (1963) 445.
3 L. A. CARLSON, B. HÖGSTEDT AND L. ORÖ, *J. Atheroscler. Res.*, 3 (1963) 467.
4 K. W. WALTON, P. J. SCOTT, J. V. JONES, R. F. FLETCHER AND T. WHITEHEAD, *J. Atheroscler. Res.*, 3 (1963) 396.
5 M. F. OLIVER, *J. Atheroscler. Res.*, 3 (1963) 427.
6 J. FOLCH, M. LEES AND G. H. S. STANLEY, *J. Biol. Chem.*, 226 (1957) 497.
7 J. JURAND AND F. ALBERT-RECHT, *Acta Clin. Chim.*, 7 (1962) 522.
8 L. A. CARLSON, *J. Atheroscler Res.*, 3 (1963) 334.
9 W. STOFFEL, F. CHU AND E. H. AHRENS, *Anal. Chem.*, 31 (1959) 307.